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The Distribution and Treatability of Perchlorate in Groundwater Baldwin Park Operable Unit San Gabriel Basin

Prepared for

Baldwin Park Operable Unit Steering Committee

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1.0 INTRODUCTION

For the past several years the Baldwin Park Operable Unit Steering Committee (BPOUSC), the U.S. EPA Region IX, Three Valleys Municipal Water District (TVMWD), and the Metropolitan Water District of Southern California (MWD) have been planning a combined groundwater remediation and water supply project in the San Gabriel Basin. Project planning was initiated in response to a requirement of U.S. EPA Region IX to remediate a plume of volatile organic compounds (VOCs) in groundwater distributed from locations north of Interstate 210 in the City of Azusa southwest to locations in the vicinity of Interstate 10 in the City of Baldwin Park. This area is called the Baldwin Park Operable Unit (BPOU).

To achieve multiple objectives, the current project combines groundwater remediation, water supply, and conjunctive use. This project is designed to extract groundwater contaminated with VOCs in two areas, treat the extracted water and deliver the treated water to water supply systems. Pumping would need to occur throughout most of the year. As the BPOUSC has no water rights in the basin; the volume of water extracted would need to be recharged. Recharge of replacement water could occur when seasonally available water could be purchased. Extraction would occur at a consistent rate throughout the year, but recharge could occur during a portion of the year, or could be postponed from one year to the next depending upon water levels in the basin or the availability of water for recharge. Therefore, the project has a conjunctive use component and as such, affords drought protection for those receiving the treated water.

The BPOUSC was in the process of negotiating agreements with the U.S. EPA, MWD, and TVMWD when in June 1997 concentrations of perchlorate ion above the State of California Department of Health Services (DHS) provisional action level of 18 μg/L were found in groundwater within the BPOU and other portions of the San Gabriel Basin. Additionally, perchlorate has recently been detected in groundwater at other facilities in California.

Before the project can move forward, the potential impact that perchlorate has on the conceptual project design must be evaluated. The presence of perchlorate in BPOU groundwater is particularly troublesome for several reasons. First, there is insufficient information known about the long-term effects of low concentrations of perchlorate on human health. Therefore, based on evaluations by U.S. EPA and DHS, a very conservative "provisional" action level with a 300-fold margin of safety has been established. As appropriate toxicological studies are completed, this action level could change significantly.

Second, there is no treatment technology that has been demonstrated to be effective in reducing concentrations of perchlorate ion in water to acceptable levels. Pilot-scale testing has been successfully performed at another site. However, the pilot test at this other site was conducted at a flow rate equivalent to 0.1% of that needed in the BPOU, the influent perchlorate concentration was over 100 times that expected in the BPOU, and the pilot system was not designed to achieve nor did it achieve effluent perchlorate concentrations less than the 18 μg/L provisional action level.

Third, the U.S. EPA Record of Decision (ROD) for the BPOU was issued in 1994, long before the discovery of perchlorate in BPOU groundwater and the establishment of the 18 μg/L provisional action level by DHS. Consequently, the current remedy proposed for the BPOU, specifically the groundwater extraction and treatment system, does not address perchlorate as a chemical of concern. In addition, the ROD does not address delivery or recharge of treated water with concentrations of perchlorate above the DHS provisional action level.

The purpose of this paper is to summarize the current understanding regarding the distribution of perchlorate in the BPOU, estimate concentrations that would be present in the effluent from the currently conceived Central Treatment Plant, review available information on the characteristics and treatability of perchlorate, and make recommendations regarding the need for treatability testing.

To resolve the issue of how the presence of perchlorate affects the BPOU project, several critical activities must be completed. These include:

- better definition of the distribution of perchlorate in BPOU groundwater
- evaluation of the current extraction plan considering the distribution of perchlorate
- better estimation of flow-weighted concentrations of perchlorate that would be expected in extracted groundwater
- refined evaluation of available information on the treatability of perchlorate in water
- performance of treatability testing to ensure BPOU groundwater can be treated to performance standards and to develop design criteria for a full-scale plant.

These activities must be undertaken before negotiations with project stakeholders (TVMWD, DHS, MWD, and others) can be completed.

2.0 THE DISTRIBUTION OF PERCHLORATE IN BPOU GROUNDWATER

Perchlorate was first detected in San Gabriel Basin groundwater in late May 1997 by the California Department of Health Services (DHS). This prompted the Main San Gabriel Basin Watermaster (MSGBWM) and the BPOUSC to perform additional groundwater sampling and analysis to better understand the distribution of perchlorate in groundwater.

To date, the BPOUSC has compiled perchlorate data from over 50 monitoring wells, production wells, and sampling points in the vicinity of the BPOU. Perchlorate analysis for production wells was performed on samples obtained by the DHS and MSGBWM and provided by the San Gabriel Basin Water Quality Authority (SGBWQA). Groundwater samples from monitoring wells in the BPOU were collected by Camp Dresser McKee (CDM), Harding Lawson Associates, and Geosyntec on behalf of the BPOUSC. All available data on perchlorate concentrations in groundwater in the San Gabriel Basin are provided in Attachments 1 and 2.

Based on these initial data, the approximate lateral distribution of perchlorate in groundwater at concentrations greater than the DHS provisional action level of 18 μg/L is illustrated on Figure 2-1. This approximate perchlorate distribution is based on maximum concentrations detected in any sample or at any depth within a given well. Maximum concentrations are posted next to each well location on Figure 2-1. Similarly, the approximate vertical distribution of perchlorate in groundwater is illustrated on the cross section shown on Figure 2-2. This cross section is oriented along the generalized direction of groundwater flow within the BPOU as shown on Figure 2-1.

Water quality results from both pre-design multiport wells and water supply production wells are plotted together on Figures 2-1 and 2-2. Production wells draw water from large screened intervals (greater than 100 feet), and therefore, represent an integrated water sample from a significant portion of the aquifer. Multiport wells have small screen intervals and therefore, represent water quality from a discrete depth interval. These data are therefore not directly comparable though comparison of these data was necessary for this presentation.

It should be noted that for the majority of these wells, only a single sample has been collected and results of quality control sampling have not been fully processed. Further, the extent of perchlorate both upgradient and downgradient of the BPOU has not been fully defined, and areas beyond the immediate boundaries of VOCs detected in the BPOU have not been sampled. Therefore, the known distribution may change as wells are resampled or new wells are sampled.

3.0 UNCERTAINTIES

In February 1997 perchlorate was discovered in five drinking water supply wells in Sacramento, California. This discovery was a result of the improvement in the method of perchlorate analysis which has only recently allowed detection of perchlorate in water at concentrations below the level which EPA and DHS considers acceptable for use by the public (18 μg/L). Because modifications to the laboratory method are recent, only one laboratory has received DHS approval to perform perchlorate analyses.

In addition, there is limited information on the long-term effects of low concentrations of perchlorate on human health and, therefore, only a "provisional" action level is available. Although additional studies on the toxicity of perchlorate are underway, results of these studies will not be available for approximately one year. As stated in Section 2.0, our understanding of the distribution of perchlorate in groundwater in the San Gabriel Basin is based on a single round of sampling a combination of water supply and monitoring wells.

In summary, there is a high level of uncertainty associated with our current understanding of the distribution of perchlorate in BPOU groundwater and whether these concentrations would pose any concerns to the public should extracted water from the BPOU be introduced into a public water supply.

3.1 Toxicity/Provisional Action Level

One of the sources of uncertainty associated with the potential effect that concentrations of perchlorate ion in groundwater may have on the selection of a remedy for the BPOU is the limited data available on the toxicity of perchlorate (at low concentrations) to humans. Because limited animal studies have been performed and no studies documenting human effects at low concentrations are available, the provisional Reference Dose (RfD) and provisional action level established by DHS includes a conservative safety factor and therefore may be subject to significant change when more toxicological data are available and other studies have been conducted.

The primary human health concern related to perchlorate is that it interferes with the thyroid gland's ability to utilize iodine to produce thyroid hormones. Perchlorate in high doses (mg/kg per day levels) has been used as a medicine to treat Grave's disease, a condition in which excessive amounts of thyroid hormone are produced. These dosage levels are much higher than would be expected from the ingestion of relatively low concentrations of perchlorate in groundwater in the San Gabriel Basin. In addition, perchlorate is used in Europe to counteract the side effects of the heart drug, amiodarone. As expected, although the health effects of high dosages of perchlorate are documented, no studies have examined the health effects at dosages potentially received from the ingestion of groundwater at concentrations present in the San Gabriel Basin groundwater. A literature search performed by the Sutter/CHS Health Resource Center Library on the health effects of perchlorate use in the treatment of Graves Disease and to counteract the side effects of amiodarone is provided in Attachment 3.

In December of 1992, the U.S. EPA National Center for Environmental Assessment (NCEA) responded to a request by U.S. EPA Region IX to evaluate the toxicity of perchlorate in soil and groundwater. Based on limited data on the toxicity of this ion, NCEA recommended a provisional RfD for soil and groundwater that included a conservative safety factor and correlated with acceptable levels of 70 mg/L and 3.5 μg/L, for these media, respectively. NCEA later stated in a letter dated February 25, 1997, that these provisional RfDs were merely opinions provided to EPA regional officials and were not to be considered formal EPA policy.

In April of 1993, a Perchlorate Study Group (PSG) was formed by the U.S. Air Force, various aerospace companies, and the two primary manufacturers of perchlorate compounds. The mission of the PSG was to review and evaluate information on the toxicity of perchlorate and develop better information on what constitutes an acceptable level of perchlorate in soil and groundwater.

In June 1995, the PSG submitted a position paper to the U.S. EPA presenting the groups' findings. The U.S. EPA again reviewed available toxicological data on perchlorate and concluded

that although information was available on the short-term effects of high concentrations of perchlorate on the thyroid, there was not enough information on the effects of long-term exposure. In October 1995, the U.S. EPA responded to the PSG paper by recommending a provisional reference dose correlating to an acceptable level in groundwater that ranged between 3.5 and 17.5 μg/L. Because there was limited information available, the U.S. EPA recommendation includes a large margin of safety. In fact a 300-fold margin of safety above the level at which no health effects were observed was used to establish the 17.5 μg/L provisional standard. This value became the 18 μg/L value currently used as the DHS provisional action level. Once there is sufficient information available, a permanent standard is expected to be established.

In March 1997, the PSG assembled a technical Peer Review Panel of nationally recognized scientists to evaluate the health effect of perchlorate in drinking water. The conclusion of this panel was that there are insufficient toxicological data available to establish a technically defensible RfD or support the U.S. EPA provisional RfD.

In May 1997, the Air Force brought the Peer Review Panel back together with California state and federal regulators in Cincinnati, Ohio. The purpose was to have the panel develop a protocol and the scope of studies that would lead to a recommendation to U.S. EPA for a new RfD which could serve as the basis for a groundwater MCL. The PSG has undertaken to commence the necessary studies in August 1997, interpret the data, peer-review the results, and submit recommendations to U.S. EPA by July 1998.

Attachment 4 contains information compiled by the PSG relative to a RfD for perchlorate. It should be noted that to date the U.S. EPA has not endorsed the Peer Review Panel but did have representatives participate on the panel. Further, U.S. EPA has not endorsed the evaluation process or committed to a schedule for review of the resultant recommendations or its effect on the U.S. EPA's former provisional RfD. As a result it is uncertain how long it will take for the provisional RfD to be revised and an MCL established.

In February 1997 the DHS set a provisional action level for perchlorate in groundwater at 4 μg/L, but at that time laboratory methods were not designed or approved to measure concentrations this low. In May of 1997 DHS, based on the results of U.S. EPA's recommendations, revised its provisional action level from 4 μg/L to 18 μg/L stating that it had reevaluated scientific studies in greater detail and had determined that 18 μg/L is consistent with the range of perchlorate exposures the U.S. EPA considers protective of human health. DHS requires that water suppliers promptly notify customers whenever perchlorate is present in concentrations greater than 18 μg/L.

3.2 Analytical Methodology and Detection Limits

At the time that the U.S. EPA set its provisional RfD and the DHS set its provisional action level for perchlorate in groundwater, no EPA laboratory method existed and few laboratories were set up to analyze for perchlorate. Some laboratories were using a modification of EPA Method 300 (Ion Chromatography), while others were using an Ion Selective Electrode (ISE). Detection limits for analysis of perchlorate in water were generally in the range of 400 to 1,000 μg/L.

It was not until April 1997, that the DHS attained the current reporting limit of 4 μg/L after having performed its own method development (Sanitation and Radiation Laboratories Branch). To date, this method has not be peer reviewed. Because perchlorate is not a regulated substance DHS does not issue laboratory certification for method analysis. DHS will however issue informal approval to perform perchlorate analysis once a laboratory meets DHS requirements.

To receive DHS approval the laboratory must hold a current certification for EPA Method 300, develop a Standard Operating Procedure (SOP), determine its Method Detection Limit (MDL), and prepare a data package demonstrating its ability to perform the analysis. The laboratory must then contact the DHS who will send out a field auditor. The laboratory must perform analysis on the samples with acceptable results (±10%) in the presence of the auditor. In June 1997, WECK Laboratories, City of Industry, California, became the first laboratory to receive DHS approval. Although other laboratories perform perchlorate analyses none has yet received DHS approval.

Because developments in analytical chemistry have only recently allowed laboratories to achieve

a MDL below the DHS provisional action level, there is a high level of uncertainty associated with analytical results. This uncertainty is compounded by the fact that most laboratories performing perchlorate analyses have modified EPA Method 300 in different ways. Table 3-1 documents the difference in analytical methodology between three commercial laboratories (California Laboratory Services, APPL, and WECK), Aerojet General Corporation's (Aerojet) in-house laboratory, and the DHS Sanitation and Radiation Branch Laboratory in Berkeley, California. It should be noted that other industrial and commercial laboratories also perform perchlorate analyses. Although all five laboratories surveyed use a modified EPA Method 300, there are substantial differences in sample storage, holding time, calibration, effluent solution, injected sample size, and column types.

Until the method has been more widely approved, a higher than normal level of quality control precautions should be taken. In conformance with this recommendation, the recent round of sampling performed by the BPOUSC included analysis of split samples for 20 percent of the wells sampled. These splits were analyzed at one primary laboratory (WECK), and two secondary laboratories (APPL and California Laboratory Services). Although results for split samples sent to California Laboratory Services are encouraging in that they generally provide a reasonable range of confirmation, all quality control data have not yet been received or processed.

Table 3-1. Summary of Perchlorate Analytical Methods

Laboratory	Cost	Turn-around Time	Matrix tested on	Bottle	Preservation	Holding Time	Sample Size	Type of Method	Reporting Limit	Method Detection Limit (MDL)	Calibration Range	Initial Calibration	Approximate Retention Time
State of California Dept. of Health Services Sanitation and Remediation Laboratory Berkeley, CA	$100.00	10 days	Drinking, Groundwater	plastic or glass	Store 4° C	28 days	125 ml	Ion Chromatography	4 µg/L	0.7 µg/L	2.5 to 500 µg/L	3 point curve	7.4 minutes
WECK Laboratories City of Industry, CA	$90.00	7 days	Drinking, Groundwater	plastic	Store 4° C	28 days	100 ml	Ion Chromatography	4 µg/L	1.6 µg/L	5 to 100 µg/L	5 point curve	9 minutes
California Laboratory Services Rancho Cordova, CA	$100.00	10 days	Drinking, Groundwater, Soil (1/1 slurry)	plastic or glass	none	14 days	250 ml	Ion Chromatography	5 µg/L	2 µg/L	5 to 500 µg/L	7 point curve	10 minutes
APPL Laboratories Fresno, CA	$80.00	10 days	Drinking, Groundwater	plastic or glass	none	14 days	250 ml	Ion Chromatography	6 µg/L	4 µg/L	5 to 500 µg/L	4 point curve	9 minutes
Aerojet General Corporation Rancho Cordova, CA	-	-	Groundwater	plastic	Store 4° C	28 days	125 ml	Ion Chromatography	400 µg/L	35 µg/L	5 to 100 µg/L	5 point curve	6.5 to 7.5 minutes

Table 3-1. Summary of Perchlorate Analytical Methods

Laboratory	Method QA/QC	Stock Standard	Eluent Solution	Equipment Brand	Injector loop	Guard Column	Separator Column	Anion Supressor
State of California Dept. of Health Services Sanitation and Remediation Laboratory Berkeley, CA	Similar to SW-846 methods or Method 300	1000 mg/L $KClO_4$ (>99% pure $KClO_4$)	120 mM 50% (w/w) NaOH, 2.0 mM p-cyanophenol (NaOH solution fresh with min. of CO_2)	Dionex	740 µL	Dionex IonPac AG5	Dionex IonPac AS5	Dionex AMMS-II
WECK Laboratories City of Industry, CA	Similar to Method 300.0 (LCS, MS/MSD, PE)	reagent grade $NaClO_4$	Sodium hydroxide with modifier	Dionex	1 ml	Dionex IonPac AG5	Dionex IonPac AS5	Dionex AMMS-II
California Laboratory Services Rancho Cordova, CA	Similar to Method 300.0 (LCS, MS/MSD)	Primary and independent source $KClO_4$	Not Provided[1]	Not Provided[1]	Less than DHS	Not Provided[1]	Not Provided[1]	Not Provided[1]
APPL Laboratories Fresno, CA	Similar to Method 300.0 (LCS, MS/MSD, Continuing Calibration Std)	$KClO_4$, $NaClO_4$ (Independent confirmation standard)	Similar to DHS method - They have made minor changes to the strengths of solutions	Not Provided[1]	Similar to DHS[1]	Similar to DHS[1]	Similar to DHS[1]	Similar to DHS[1]
Aerojet General Corporation Rancho Cordova, CA	Similar to Method 300.0 (LCS, MS/MSD)	$NaClO_4$, check with $KClO_4$	900 ml 100 mM NaOH; 800 ml methanol, dilute to 3 L	Dionex	100 µL	Dionex AG11	Dionex AS11	Dionex ASRS-1

1 The laboratory considered the information proprietary and did not provide specific information

Table 3-1. Summary of Perchlorate Analytical Methods

Laboratory	Detector	Interferences	Miscellaneous Requirements/Other Notes
State of California Dept. of Health Services Sanitation and Remediation Laboratory Berkeley, CA	Conductivity	No specific species noted High concentrations of other anions may interfere	1) Method tested for sample with conductivity up to 1000 µmhos/cm. Method has not been tested for conductivities >1000 µmhos/cm 2) Fortification method recommended for unknown samples (appears that this is just a matrix spike sample. Analyzed to evaluate if the sample matrix will bias analytical results) 3) p-cyanophenol added to eluent to deactivate the active sites on the resin
WECK Laboratories City of Industry, CA	Conductivity	None expected. High concentrations of other anions may interfere	Approved by State of California DHS to analyze for perchlorate ion in drinking water (June 20, 1997) Would do split to analyze for chlorate
California Laboratory Services Rancho Cordova, CA	Conductivity	None expected. High concentrations of other anions may interfere	3-4 months experience with method on field samples
APPL Laboratories Fresno, CA	Conductivity	None expected. High concentrations of other anions may interfere	1-2 months experience with the method
Aerojet General Corporation Rancho Cordova, CA	Conductivity	None expected. High concentrations of other anions may interfere	

4.0 TREATABILITY REVIEW

Treatability information on perchlorate in water was obtained primarily from two sources. The first source consists of recent data collected by the MSGBWM and the DHS in conjunction with groundwater sampling in the San Gabriel Basin as described in Section 2. The second source was obtained from treatability studies conducted by Aerojet at their Sacramento facility.

4.1 Existing Treatment System Data

The MSGBWM and the DHS collected samples at several groundwater treatment systems in San Gabriel Basin in conjunction with groundwater sampling from production wells. Perchlorate concentrations in influent and effluent streams for these treatment systems are provided in Attachment 2. As expected, no apparent reduction in perchlorate concentration was observed at those treatment systems utilizing air stripping techniques. However, perchlorate concentrations at two granular activated carbon (GAC) treatment units located at the Valley County Water District Lante and Big Dalton wells suggest that perchlorate concentrations are reduced as extracted groundwater passes through GAC contactors. However, these data are inconclusive as the trends initially observed were not reproduced in subsequent sampling events. Nonetheless, some reduction in perchlorate concentration may be occurring.

At this time the mechanism causing this apparent reduction in perchlorate concentration is unknown. Two possible mechanisms are: 1) the direct adsorption of perchlorate onto GAC, and 2) biochemical reduction by naturally occurring microorganisms that are present in carbon vessels.

If decreases in perchlorate concentration are due to adsorption, fresh carbon would provide the greatest perchlorate removal. Carbon that has been in service for some time would provide less capacity for perchlorate removal and in fact may eventually desorb perchlorate, resulting in concentrations that are higher in the effluent than observed in the influent.

If the mechanism which is responsible for the apparent decrease in perchlorate concentrations is biochemical reduction, GAC units containing fresh carbon would not be expected to remove perchlorate as efficiently as carbon that had been in service for some time because populations of microorganisms would not yet be established. Treatability studies performed in Sacramento have demonstrated that GAC is a superior medium for growth of perchlorate reducing microorganisms.

Dates of carbon changeout will be obtained and this issue will be reviewed more carefully as part of recommended treatability studies described in Section 6.

4.2 Aerojet Treatability Studies

As a result of the presence of perchlorate in groundwater at Aerojet's Sacramento facility, a considerable amount of work has been performed to address perchlorate treatability. This work, consisting of technology screening, laboratory-scale studies, pilot-scale studies, and the design of a full-scale (1,500 gpm) system, was performed by Aerojet and a consultant starting in 1994.

4.2.1 Literature Review

In 1994, Aerojet completed an initial screening of technologies available for treatment of perchlorate. An on-line data search was first performed. The following databases were searched:

- Energy SciTech (1974-1994)
- Ei Compendex Plus (TM) (1970-1994)
- National Technical Information Service (1964-1994)
- Aerospace Database (1962-1994)
- Chemical Engineering Abstracts (1970-1994)
- Biotechnology Abstracts (1970-1994)
- PTS Aerospace/Defense Markets (1986-1994)
- Pollution Abstracts (1970-1994)
- Analytical Abstracts (1980-1994)

Only limited information on the treatment of water for perchlorate was found, and the available data addressed the treatment of high concentration wastewaters, not low concentrations in groundwater. The technologies for which information was found include both biological and physical/chemical treatment methods.

Biological Methods

Biochemical reduction of oxygen-containing compounds, like perchlorate, with the simultaneous biochemical oxidation of organic matter contained in sludge from municipal wastewater treatment plants was the subject of three patents (1973-1994). The patents varied in reactor configuration and the source and type of the microorganisms used. Concentrations of perchlorate in wastewater in excess of 7,000 mg/L were the subject of these patents.

A 1973 patent describes biochemical oxidation of activated sludge in an unaerated tank. A 1976 patent is a modification of this approach but a specific microorganism is identified. The source of the microorganism is settled municipal sewage. A 1994 patent by the U.S. Air Force uses an anaerobic reactor and a specific microorganism. Brewer's yeast, cottonseed protein, and whey powder were all added to the reactor.

Physical/Chemical Methods

The physical/chemical processes which were reviewed included ion exchange, reverse osmosis, an electrochemical process which reduces inorganic oxyhalides, and a process where perchlorate wastewater was treated with an oxidant in supercritical (high temperature, high pressure) water.

The electrochemical method, patented in 1992, uses an anode/cathode separated by a cation exchange membrane. A 1993 paper describes treatment of perchlorate in wastewater with an oxidant (O_2, air, H_2O_2) under conditions of high pressure (200 atm) and temperature (370°C).

In addition to these two techniques, Aerojet's staff reviewed the applicability of ion exchange and reverse osmosis treatment technologies. Although both ion exchange and reverse osmosis are considered technically proven methods for reducing concentrations of dissolved solids in waters, there are significant technical challenges presented by both methods for treatment of water containing perchlorate.

With respect to ion exchange, common groundwater ions will interfere with perchlorate adsorption. The ion exchange resin is regenerated with brine (usually sodium chloride). Perchlorate concentrations in regeneration brine present a unique disposal or treatment problem.

There are significant operational difficulties associated with the use of reverse osmosis. Like ion exchange, perchlorate is not treated but merely conveyed to a waste concentrate that would be a waste disposal challenge. The resultant brine would contain perchlorate and would be significant in volume. In addition, pretreatment of influent, use of anti-fouling chemicals, and membrane cleaning are time-consuming and costly.

Based on the literature review described above, Aerojet decided to pursue laboratory-scale testing of chemical reduction and biochemical methods.

4.2.2 Laboratory-Scale Testing

Laboratory-scale treatability studies for several biochemical and chemical reduction treatment methods were performed by an Aerojet consultant in 1995. The tested water came from Aerojet's Sacramento facility and contained between 7,000 and 8,000 μg/L perchlorate.

Chemical reduction methods evaluated included the addition of relatively high dosages of several reducing agents (sodium sulfite, sodium bisulfite, and sodium thiosulfate) up to 1,000 mg/L to water containing 7,000 μg/L perchlorate. Perchlorate concentrations did not significantly decrease over time. The method was concluded to be ineffective, and not taken to pilot-scale.

In addition to chemical reduction, Aerojet staff evaluated the use of ion exchange technology in more detail. Time was devoted to resin selection and treatment of regeneration wastes. Efforts were also made to develop a method for biodegradation of perchlorate in these wastes. Aerojet is presently evaluating the viability of ion exchange technology for perchlorate removal, specifically for use at wells where concentrations are 100 μg/L or less.

Two biochemical reduction methods were tested: a fixed film bioreactor using submerged plastic media, and a granular activated carbon/fluidized bed (GAC/FB). For both processes the water to be treated was amended with an organic carbon source (acetate or ethanol) and nutrients (nitrogen and phosphorus) before entering the bioreactor.

Both biochemical reduction methods were shown to be effective in reducing perchlorate concentrations. The GAC/FB system was better at responding favorably to system changes and also accommodated a higher (6-fold) perchlorate loading rate. Effluent from both processes were below a 400 μg/L reporting limit for perchlorate.

Because of the success with the biochemical treatment methods, and due to the comparatively better performance of the GAC/FB method, this method was taken to pilot-scale.

4.2.3 Pilot-Scale Testing

In 1996, a 30 gpm skid-mounted pilot system, was set up at the Aerojet facility in Sacramento.

The pilot-scale system operated between April and December of 1996. Operation of this pilot-scale system allowed optimization of feed rates for the organic carbon source (ethanol) and nutrients (nitrogen in the form of ammonium chloride and phosphorus in the form of dibasic sodium biphosphate). Ethanol was required in an ethanol to perchlorate molar ratio of approximately 4:1, while nitrogen and phosphorus levels were similar to those described in the literature to support microbial activity.

Effluent concentrations were consistently less than a 400 μg/L reporting limit for perchlorate, 500 μg/L for phosphorus, 340 μg/L for ammonia-nitrogen, and less than 50 μg/L for nitrate-nitrogen.

The initial pilot-scale effluent contained very low or nondetectable levels of E. coli bacteria. After one month of operation, all E. coli measurements showed nondetectable levels. Regardless, disinfection of treatment system effluent was envisioned for a full-scale system.

4.2.4 Full-Scale Design

Aerojet is in the process of designing a full-scale perchlorate treatment system for one of the groundwater extraction and treatment systems at their Sacramento facility. The design is expected to be complete in July 1997, and construction is currently scheduled to be complete in the summer of 1998. The system loading rate is 1,500 gpm. The full-scale system will be similar to that pilot-tested in 1996.

Aerojet is working with the design contractor to optimize certain design features and to lower effluent concentrations. The pilot-scale study was completed prior to the recent reduction in MDLs by agency and commercial laboratories and, therefore, Aerojet and its contractor are hoping to modify either the design or operating parameters to produce effluent below the 18 μg/L provisional action level.

In addition, Aerojet and its contractor have evaluated alternative sources of microorganisms to eliminate possible problems with the potential introduction of pathogens associated with wastewater treatment plant sludge. Waste sludge from a baby food processing facility was determined to contain acceptable microorganisms thus eliminating the potential for pathogens to be present in treatment effluent.

5.0 APPLICABILITY OF TREATABILITY STUDIES TO THE BPOU

The extent to which treatability studies performed at Aerojet's Sacramento facility can be relied upon to assist in decision-making on the BPOU project is dependent upon similarity in the composition of influent water, effluent goals, and the capacity of the treatment system.

5.1 Influent Water

Although there is insufficient data at this time to estimate with reasonable confidence a concentration that would represent influent to the Central Treatment Plant, a rough estimate for planning purposes was considered necessary. Therefore, based on the distribution of perchlorate in groundwater described in Section 2.0, the configuration of extraction wells and flow rates described in the December 1996 Pre-Remedial Design Report, and modifications to the extraction plan discussed with EPA, it has been estimated that extracted groundwater would contain approximately 50 to 100 μg/L of perchlorate. This range of values was estimated by selecting surrogate wells for each extraction well location, assigning recently measured concentrations from each surrogate well to its corresponding extraction well, and flow-weighting these concentrations based on expected pumping rates to produce a flow-weighted average concentration for the BPOU extraction system. Because of the uncertainty associated with this type of estimate, a concentration range was established.

It should be noted that there are significant uncertainties associated with the 50 to 100 μg/L range in concentrations cited above. First and most important, the current extraction plan does not take into account the distribution of perchlorate in groundwater. Should capture of groundwater containing perchlorate become a remedial action objective, then both the locations and pumping rates of BPOU extraction wells may need to be modified. Additionally, the method described above is a rough estimation of concentrations that will be initially extracted. The actual concentrations present in the extracted groundwater will only be know once extraction wells are constructed and pumped at their designed flow rates.

The primary difference between the composition of influent water quality in Sacramento and the BPOU is the concentration of perchlorate. Concentrations of perchlorate in Sacramento range from 7,000 to 10,000 μg/L with individual onsite wells having concentrations as high as 100,000 μg/L. The expected flow-weighted average from the BPOU extraction system as currently configured is estimated to be approximately 50 to 100 μg/L.

These variances may cause a difference in system performance. A potential concern exists that at a lower influent concentration, microorganisms that reduce perchlorate will not flourish in sufficient numbers. As the microorganisms rely on ethanol as their primary food source and perchlorate as a secondary food source, this should be evaluated, but may not be of concern.

5.2 System Capacity

The largest pilot-scale study performed to date was operated for approximately 8 months at 25 gpm. This is not a sufficient flow rate to allow design of a system that will operate at 20,000 gpm. The full-scale system presently in design at Aerojet's Sacramento facility may be of sufficient capacity (1,500 gpm) to allow scale up to 20,000 gpm, but this system will not be in operation until the summer of 1998.

5.3 Effluent Goal

When the pilot-scale study was performed at Aerojet's Sacramento facility, the goal was to produce effluent that was less than the 400 μg/L laboratory reporting limit available at that time. When the pilot-scale study was completed, the effluent generally was characterized by perchlorate concentrations less than 100 μg/L. At that time is was not possible to measure to the current reporting limit of 4 μg/L for perchlorate. Similarly, it was not possible to optimize system flow rates, organic carbon sources, or nutrients to see if lower effluent concentrations were possible. Therefore, it is uncertain if the full-scale system to be constructed by Aerojet in Sacramento may reach treatment goals for the BPOU. Treatability studies will need to demonstrate that a sufficiently low

perchlorate concentration in treatment plant effluent is possible.

5.4 Nitrate Concentrations

Influent groundwater at Aerojet's Sacramento facility is characterized by low (1.5 mg/L) nitrate concentrations. However, the results of the pilot-scale study performed in Sacramento do show effluent nitrate concentrations less than 0.05 mg/L. This suggests that along with consumption of ethanol and reduction of perchlorate, reduction of nitrate is also occurring in the bioreactor.

This observation could have a significant effect on the BPOU project as influent nitrate concentrations are expected to be approximately 25 mg/L. Although this concentration is well below the 45 mg/L MCL, it is substantially higher than concentrations currently received by customers of MWD and TVMWD.

Supporting evidence that the same anoxic conditions which contribute to the reduction of perchlorate may also reduce nitrate concentrations may be found in the literature where processes using bacterial denitrification of wastewater have been described. Although the denitrification process has generally not been applied to drinking water, one such system was designed for the town of Wiggins, Colorado to denitrify its drinking water. The process equipment, designed by Joann Silverstein of the University of Colorado, Boulder, consists of a packed tower biofilm reactor where denitrifying bacteria are supported on a high-porosity plastic media. The packed tower is operated in an up-flow mode. Bacteria were introduced into the system from creek sediments, and acetic acid and corn syrup were used as the organic carbon source. The denitrified water was then passed through a sand filter to remove biomass. The system operates at a flow rate of 10 gpm. Influent nitrate-nitrogen concentrations were initially 7 to 10 mg/L but increased to 25 mg/L. During initial start-up and operation, system performance was not satisfactory and it was determined that both nitrogen and phosphorus were needed as nutrients to optimize nitrate removal efficiency. Effluent now contains 2 mg/L nitrate-nitrogen.

Should the GAC/FB system prove to be an effective method of reducing nitrate concentrations in treatment plant effluent, it may be possible to reduce both perchlorate and nitrate concentrations.

6.0 RECOMMENDATIONS

In order to proceed with the design and construction of the BPOU project, several key issues related to perchlorate must first be resolved. These issues include:

- The need for a more definitive action level for perchlorate and consideration of this action level relative to the remedial action objectives specified in the ROD
- The potential need for modifications to the current extraction plan and the conceptual design of the Central Treatment Plant, if groundwater extraction and treatment of perchlorate is deemed necessary
- The need for additional treatability studies to address the feasibility of perchlorate treatment at high flow rates and low concentrations.

As a result of these issues, the following three recommendations have been developed:

- The remedial action objectives in the ROD for the BPOU should be reconsidered to include an appropriate, scientifically supportable action level for perchlorate
- Proposed groundwater extraction and treatment plans should also be reconsidered in the event that remedial action objectives are revised to address perchlorate
- Recommended treatability studies on perchlorate treatment in water should be performed as described below.

6.1 Recommended Treatability Testing

Because there is no proven treatment technology for perchlorate, the need to add perchlorate treatment to the Central Treatment Plant is undetermined until EPA includes perchlorate as a chemical of concern in the BPOU ROD. Further the feasibility of perchlorate treatment at the scale required for the BPOU can not be determined until treatability studies can be completed. As laboratory- and pilot-scale testing have been performed to support design of a full-scale system at Aerojet's Sacramento facility, it would be expedient and wise to take advantage of this work in resolving San Gabriel Basin issues. However, as discussed earlier, conditions in Sacramento are different and therefore testing to allow design of a San Gabriel Basin system is necessary.

There are several key differences that should be considered in treatability testing to support the BPOU project. These include:

- a lower perchlorate influent concentration
- a lower effluent goal for perchlorate
- higher nitrate influent concentrations
- a higher flow rate.

To collect adequate information on the treatability of perchlorate to meet the needs of the BPOU project, it is recommended that a two-step treatability approach be used. While treatability testing for the BPOU project is being performed, input would be received from Aerojet's full-scale Sacramento treatment unit and results considered in the design of the BPOU system.

6.2 Phase 1 - Pilot-Scale Treatability Testing

It is recommended that the BPOUSC perform pilot-scale treatability testing in two phases. The objective of the Phase 1 testing is to ensure that the GAC/FB system already pilot-tested at Aerojet's Sacramento facility can achieve a perchlorate effluent concentration less than the DHS 18 μg/L provisional action level or whatever regulatory level is ultimately set. The scope and goal of this phase of treatability testing would be established in a Phase 1 Treatability Testing Work Plan. This Work Plan would be provided to the U.S. EPA, DHS, and MWD for approval with copies for comment to the MSGBWM and SGBWQA.

To perform this phase of the treatability testing, the pilot-scale unit previously used at Aerojet's Sacramento facility would be reassembled and testing performed at Aerojet's Sacramento facility. The system would be put into operation for 60 days treating groundwater containing perchlorate concentrations similar to that expected

in San Gabriel Basin (50 to 100 μg/L). In addition, sodium nitrate should be added to the influent water to simulate San Gabriel Basin nitrate concentrations. To simulate San Gabriel Basin treatment plant conditions, VOCs would be removed by air stripping prior to flow into the bioreactor.

Testing procedures would be specified in the Phase 1 Treatability Testing Work Plan, but in general would follow procedures used at Aerojet's Sacramento facility. Regular monitoring of dissolved oxygen, total dissolved solids, common ions, total suspended solids, pH, redox potential, perchlorate, nitrate, and other parameters would be performed for both treatment plant influent and effluent. Recently developed laboratory methods will be used to assure the analytical reporting limit is less than the 18 μg/L provisional action level.

Should the Phase 1 pilot-scale testing demonstrate that effluent goals can be met, the treatability testing would move to a Phase 2 stage. Following completion of Phase 1 testing, test results would be compiled, evaluated, and submitted to EPA, DHS, and MWD in a brief report with copies also to the MSGBWM and SGBWQA. This report would also include recommendations for the implementation of Phase 2 treatability testing, if deemed appropriate.

6.3 Phase 2 - Pilot-Scale Treatability Testing

This phase of treatability testing would consist of the use of a pilot system very similar to that assembled at Aerojet's Sacramento facility but on a larger scale. The objectives of this portion of the pilot-scale testing are to allow direct treatment of San Gabriel Basin groundwater from a well or near a well location that would be part of the BPOU extraction system and to operate this system at a flow rate of 100 gpm or greater.

If arrangements for delivery of large volumes of treated water can be made, this system could be designed to operate at a hydraulic loading rate closer to that conceived in the extraction plan.

Ideally an existing groundwater supply well, like the Lante well, that has perchlorate concentrations in the range of 50 to 100 μg/L, a large holding tank, and a connection to the Valley County Water District System, could be used. Use of a facility like the Lante well site should eliminate concerns regarding discharge of treated water, as this system has been operated in this manner for some time.

This pilot system should be operated for at least three months. Regular performance monitoring would include measurement of dissolved oxygen, total dissolved solids, total suspended solids, pH, nitrogen, phosphorus, perchlorate, and redox potential, as well as nitrate and perchlorate.

To simulate BPOU conditions, the pilot system would include an air stripper to remove VOCs prior to perchlorate/nitrate treatment. The objectives and scope of the testing would be described in a Phase 2 Treatability Testing Work Plan. This work plan would be provided to the U.S. EPA, DHS, and MWD for approval with copies for comment to the MSGBWM and SGBWQA. Following completion of Phase 2 testing, test results would be compiled, evaluated, and submitted to EPA, DHS, and MWD in a brief report with copies to the MSGBWM and SGBWQA.

6.4 Aerojet Sacramento - Integration of Full-Scale System Performance Data

There is certainly much to be learned from the construction, start-up, and operation of the perchlorate treatment system that is currently undergoing design at Aerojet's Sacramento facility. Although influent concentrations are substantially higher than that anticipated in the BPOU, nitrate concentrations are substantially lower than anticipated in the BPOU, and the flow rate is less than 10 percent of that for the BPOU project, valuable information can be obtained by monitoring the performance of this system once in operation.

The Sacramento system is not expected to start operating until the summer of 1998. It is assumed that 90 days of operation will be required for the system to become fully operational, system operating parameters to be optimized, and performance data to become available. Therefore, performance data from this system will not be available as input to the BPOU project until the fall of 1998.

It is expected that the results from operation of this system will provide the BPOUSC with information on:

- efficiency of perchlorate reduction
- feed rate for ethanol
- required nutrient feed rates
- factors influencing microorganism stability
- effect on nitrate concentrations

Should the Phase 2 treatability studies be constrained by disposal of treated water, performance data from this full-scale system can be used to design the BPOU system.

6.5 Schedule for Treatability Studies

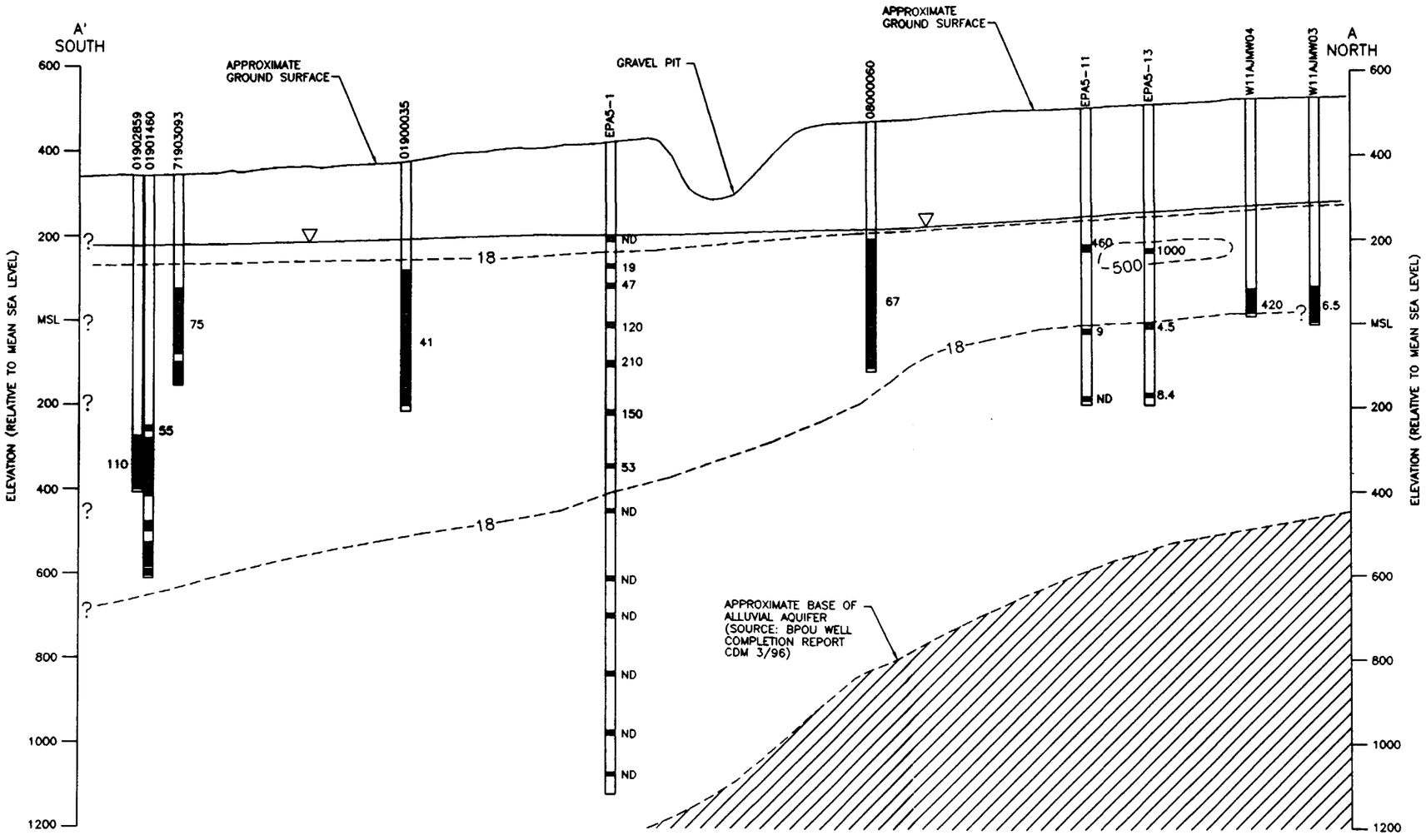
Assuming the U.S. EPA agrees with the approach to treatability testing described above, the BPOUSC will prepare a Phase 1 Treatability Testing Work Plan. This Work Plan could be ready for U.S. EPA, DHS, and MWD review and comment in late August 1997. Assuming minimal time is required to address Work Plan comments, Phase 1 treatability testing could be started in October 1997 with results available in March 1998. Assuming Phase 1 results demonstrate effluent goals can be met, Phase 2 testing could commence with EPA, DHS, and MWD approval in April 1998.

Phase 2 testing could be completed in September 1998, about the same time that data will be available from Aerojet's Sacramento perchlorate treatment unit. Input from both phases of treatability testing and performance data from Aerojet's Sacramento treatment unit would provide the BPOUSC with the necessary information to evaluate the feasibility of perchlorate treatment in the BPOU.

A listing of tasks, durations, and approximate completion dates for proposed treatability testing activities is provided below:

Proposed Schedule for Treatability Studies

Task	Duration	Approximate Completion Date
Phase 1 Treatability Testing Work Plan	30 days	09/01/97
EPA, DHS, and MWD Review and Approval	30 days	10/01/97
Phase 1 Treatability Testing Implementation	90 days	01/01/98
Phase 1 Reporting with Phase 2 Treatability Testing Work Plan	60 days	03/01/98
EPA, DHS, and MWD Review and Approval	30 days	04/01/98
Phase 2 Treatability Testing Implementation	150 days	09/01/98
Phase 2 Reporting	60 days	11/15/98



EPA5-13 — WELL ID NUMBER

PLANK CASING

PERFORATED CASING

39 — DEPTH-SPECIFIC PERCHLORATE CONCENTRATION

---18--- ESTIMATED LINE OF EQUAL PERCHLORATE CONCENTRATION (ug/l)

ND — NON DETECT

NOTES:

SEE FIGURE 2-1 FOR CROSS SECTION A-A' LOCATION

BASED ON DATA COLLECTED JUNE 1997 PERCHLORATE

SCALE:
HORIZ: 1" = 2000'
VERT: 1" = 200'

22087/CAD/32002001 7/11/97 RLB

Harding Lawson Associates
Engineering and Environmental Services

VERTICAL DISTRIBUTION OF PERCHLORATE CROSS SECTION JUNE 1997
Bauldwin Park Operable Unit
San Gabrel Basin, California

FIGURE **2-2**

DRAWN	JOB NUMBER	APPROVED	DATE	REVISED DATE
RLB	37087.2.3.2		7/97	

/ruby1/aerojob/emla/aero_new.aml

01902116 <4
W11AJMW5 <4
08000076 <4
W11AJMW2 790
W11AJMW3 8.5
W11AJMW4 4.20
08000075 <4
W11AJMW1 160
W11AZW03 39
MW5-13 1000
W11AZW09 90
MW5-17 39
W11AZW06 850
MW5-11 460
11900038 4.2
MW5-18 51
W11AZW01 <40
08000103 7
MW5-09 14
01900028 <4
01900027 <4
01900032 <4
01902356 <4
08000060 67
01900029 13
EPAW5101 210
01900885 <4
MW5-08 4
MW5-05 14
MW5-16 73
01900883 <4
01900035 41.1
08000069 6
01901599 11
08000092 <4
08000100 <4
01902967 <4
01902856 <4
01901460 58
08000062 159
01902859 110
01901161 <4
01903067 <4
01903061 <4
08000112 <4
01902947 <4
08000093 6
01903067 13
51900716 14
51900719 9
08000096 <4
08000099 <4
08000108 <4
01901439 6
08000077 <4

500
5
18
A
A'

N

0 2000 4000 6000 8000

Scale in feet

Explanation

- Pre-design well
- Monitoring well
- Production well
  ND = Not Detected

TCE Contour

Perchlorate Contour

Cross Section A-A'
(See Figure 2-2)

All units in ug/l.

Harding Lawson Associates
Engineering and
Environmental Services

Maximum Perchlorate Concentrations in Groundwater
June 1997
Baldwin Park Operable Unit
San Gabriel Basin, California

FIGURE
2-1

DRAWN	JOB NUMBER	APPROVED	DATE	REVISED DATE
SAS	37087 2.3.2			

Attachment 1

PERCHLORATE IN RAW GROUNDWATER - MONITORING AND PRODUCTION WELLS
(from Water Quality Authority Database)

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

SAMPLING_SOURCE	RECORDATION_NUMBER	PERFORATION	SAMPLING_DATE	SAMPLED_BY	RESULTS (PPB)
United States Environmental Protection Agency					
MW5-01	-				
	Zone 1	1496-1506	6/17/97	CDM	<4
	Zone 2	1387-1397	6/17/97	CDM	<4
	Zone 3	1256-1266	6/17/97	CDM	<4
	Zone 4	1123-1133	6/17/97	CDM	<4
	Zone 5	1030-1040	6/17/97	CDM	<4
	Zone 6	875-885	6/18/97	CDM	<4
	Zone 7	765-775	6/18/97	CDM	53
	Zone 8	640-650	6/18/97	CDM	150
	Zone 9	523-533	6/18/97	CDM	210
	Zone 10	430-440	6/18/97	CDM	120
	Zone 11	335-345	6/18/97	CDM	47
	Zone 12	287-297	6/18/97	CDM	19
	Zone 13	216-226	6/18/97	CDM	<4
MW5-03	-				
	Zone 1	1150-1160	6/24/97	CDM	<4
	Zone 2	1015-1025	6/24/97	CDM	<4
	Zone 3	920-930	6/24/97	CDM	<4
	Zone 4	810-820	6/24/97	CDM	<4
	Zone 5	670-680	6/25/97	CDM	<4
	Zone 6	590-600	6/25/97	CDM	7.8
	Zone 7	510-520	6/25/97	CDM	12
	Zone 8	400-410	6/25/97	CDM	8.5
	Zone 9	300-310	6/24/97	CDM	<4
	Zone 10	235-245	6/24/97	CDM	14
MW5-05	-				
	Zone 1	552-562	6/19/97	CDM	<4
	Zone 2	464-474	6/19/97	CDM	4.5
	Zone 3	380-390	6/19/97	CDM	7.7
	Zone 4	218-228	6/19/97	CDM	14

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

MW5-08	-				
	Zone 1	795-805	6/19/97	CDM	<4
	Zone 2	670-680	6/19/97	CDM	<4
	Zone 3	554-564	6/19/97	CDM	<4
	Zone 4	380-390	6/19/97	CDM	4
MW5-11	-				
	Zone 1	690-700	6/19/97	CDM	<4
	Zone 2	530-540	6/19/97	CDM	9
	Zone 3	310-320	6/19/97	CDM	460
MW5-13	-				
	Zone 1	684-694	6/23/97	CDM	8.4
	Zone 2	520-530	6/23/97	CDM	4.5
	Zone 3	340-350	6/23/97	CDM	1000
MW5-15	-				
	Zone 1	670-680	6/20/97	CDM	22
	Zone 2	450-460	6/20/97	CDM	73
	Zone 3	235-245	6/20/97	CDM	32
MW5-17	-				
	Zone 1	698-708	6/23/97	CDM	3.3 E
	Zone 2	540-550	6/23/97	CDM	<4
	Zone 3	305-315	6/23/97	CDM	39
MW5-18	-				
	Zone 1	780-790	6/23/97	CDM	51
	Zone 2	630-640	6/23/97	CDM	4.4
	Zone 3	500-510	6/23/97	CDM	3.8 E
Aerojet					
MW-1	-	283-333		HLA	160
MW-2	-	280.5-330.5		HLA	790
MW-3	-	303-353		HLA	6.5
MW-4	-	293-343		HLA	420

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

MW-5	-	267-317		HLA	<4
Azusa Land Reclamation (ALR)					
MW-1R	-	148-354		HLA/Geosyntec	<40
MW-2	-	145-350			
MW-3	-	180-385		HLA/Geosyntec	39
MW-4	-	350-614			
MW-6	-	195-450		HLA/Geosyntec	550
MW-8	-	195-450			
MW-9	-	195-450		HLA/Geosyntec	90
Azusa, City of					
7 (AVWC 5)	1902116	120-246	6/3/97	DHS	<4
10 (AVWC 8)	8000103	792-1132	6/397	DHS	7
10 (AVWC 8)	8000103	792-1132	6/10/97	DHS	6
California Domestic Water Company					
2	1901181	437-492	6/9/97	DHS	<4
		683-710			
		717-736			
		761-782			
3	1903057	197-785	6/6/97	MSGBWM	<4
3	1903057	197-785	6/9/97	DHS	<4
5A	8000100	197-785	6/10/97	DHS	<4
		460-660			
		700-900			
6	1902967	200-800	6/9/97	DHS	<4
8	1903081	200-580	6/9/97	DHS	<4
2	1910199	-	6/9/97	-	ND
3	1910199	-	6/6/97	-	ND
3	1910199	-	6/9/97	-	ND
County of Los Angeles					

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

Santa Fe	8000070	290-435	6/7/97	MSGBWM	NA
Covina Irrigating Company					
Bal-1	1900885	NA	6/3/97	DHS	<4
Bal-2	1900883	NA	6/3/97	DHS	<4
City of El Monte					
5 @ 145'	1901695	126-154	6/12/97	MSGBWM	5.9
		178-190			
		238-282			
		314-322			
		330-342			
5 @ 270'	1901695	126-154	6/12/97	MSGBWM	5.2
		178-190			
		238-282			
		314-322			
		330-342			
5 @335'	1901695	126-154	6/12/97	MSGBWM	5.5
		178-190			
		238-282			
		314-322			
		330-342			
10 @ 220'	1901699	235-246	6/12/97	MSGBWM	<4
		258-266			
		275-286			
		295-306			
		382-390			
		403-446			
		457-468			
		479-498			
10 @ 280'	1901699	235-246	6/12/97	MSGBWM	<4
		258-266			
		275-286			
		295-306			

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

		382-390			
		403-446			
		457-468			
		479-498			
10 @ 420'	1901699	235-246	6/12/97	MSGBWM	<4
		258-266			
		275-286			
		295-306			
		382-390			
		403-446			
		457-468			
		479-498			
12	1903137	150-570	6/12/97	MSGBWM	<4
La Puente Valley County Water District					
2	1901460	600-604	6/6/97	MSGBWM	58
		636-675			
		678-739			
		742-766			
		825-833			
		835-845			
		879-935			
		936-940			
2	1901460	600-604	6/12/97	PRODUCER	51
		636-675			
		678-739			
		742-766			
		825-833			
		835-845			
		879-935			
		936-940			
2	1901460	600-604	6/10/97	DHS	55
		636-675			
		678-739			
		742-766			
		825-833			

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

		835-845			
		879-935			
		936-940			
3	1902859	620-770	6/6/97	MSGBVWM	110
3	1902859	620-770	6/10/97	DHS	73
3	1902859	620-770	6/12/97	PRODUCER	76
4	8000062	550-725	6/6/97	MSGBVWM	60
4	8000062	550-725	6/10/97	DHS	159
Miller Brewing Company					
1	8000075	515-975	6/5/97	DHS	<4
2	8000076	NA	6/5/97	DHS	<4
San Gabriel Valley Water Company					
B4B	1902858	920-940	6/3/97	DHS	<4
		950-1154			
B4C	1902947	798-839	6/3/97	DHS	<4
		844-885			
		920-936			
		972-1022			
B5A	1900718	110-152	6/6/97	MSGBVWM	14
		173-180			
		194-205			
		235-245			
		276-299			
B5B	1900719	172-185	6/9/97	DHS	9
		236-254			
		286-302			
		328-340			
		386-408			
B5B	8000112	1013-1024	6/9/97	DHS	<4
		1110-1128			
		1177-1275			
B6C	1903093	275-420	6/3/97	DHS	74
		440-465			

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

		480-506			
B6C	1903093	275-420	6/9/97	DHS	71
		440-465			
		480-506			
B6C	1903093	275-420	6/3/97	MSGBVWM	75
		440-465			
		480-506			
B6C 1/	1903093	275-420	6/3/97	MSGBVWM	75
		440-465			
		480-506			
B6D	8000096	760-769	6/3/97	DHS	<4
		824-836			
		855-905			
		906-938			
		942-952			
		980-992			
		1024-1032			
B6D	8000096	760-769	6/9/97	DHS	<4
		824-836			
		855-905			
		906-938			
		942-952			
		980-992			
		1024-1032			
11B	1900745	178-280	6/6/97	MSGBVWM	<4
		358-400			
B11A	1901439	330-342	6/9/97	DHS	6
		350-372			
		643-675			
		822-833			
B11B	8000108	302-832	6/9/97	DHS	<4
B9B	8000099	818-830	6/9/97	DHS	<4
		855-859			
		873-885			
		921-939			
		1010-1022			
		1042-1070			

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

		1095-1100			
B7E	8000122	675-689	6/9/97	DHS	<4
		740-761			
		867-885			
		1040-1048			
		1078-1083			
		1104-1112			
		1128-1138			
		1148-1170			
B7C	8000068	280-780	6/9/97	DHS	<4
South Down (AZ-Two)					
2	1900038	350-614	6/5/97	DHS	<4
2 1/	1900038	350-614	6/5/97	MSGBVWM	4.2
Suburban Water Systems					
Big Dalton	1900035	250-582	5/29/97	DHS	41.1
Big Dalton	1900035	250-582	6/3/97	DHS	40
Big Dalton 1/	1900035	250-582	6/3/97	MSGBVWM	41
Big Dalton	1900035	250-582	6/6/97	DHS	38
Big Dalton	1900035	250-582	6/11/97	DHS	36
126W-2	8000092	200-620	6/9/97	PRODUCER	<4
139W-2	1901599	105-360	6/3/97	DHS	11
139W-4	8000069	566-642	6/3/97	DHS	6
		676-695			
		787-825			
139W-6	8000152	NA	6/3/97	DHS	<4
140W-3	1903067	150-160	6/3/97	DHS	13
		205-230			
		248-260			
		350-380			
		432-438			
140W-4	8000093	420-1190	6/3/97	DHS	6
140W-5	8000145	600-1320	6/3/97	DHS	<4
147W-3	8000077	300-1000	6/3/97	PRODUCER	<4

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

201W-4	1901433	120-192	6/9/97	PRODUCER	<4
		194-205			
		208-235			
		251-275			
		307-340			
		342-354			
		416-422			
		424-434			
		436-497			
		505-521			
		583-614			
201W-4	1901433	120-192	6/11/97	DHS	<4
		194-205			
		208-235			
		251-275			
		307-340			
		342-354			
		416-422			
		424-434			
		436-497			
		505-521			
		583-614			
201W-5	1901432	160-216	6/9/97	PRODUCER	<4
		251-264			
		300-332			
		435-472			
		480-512			
201W-5	1901432	160-216	6/11/97	HDS	<4
		251-264			
		300-332			
		435-472			
		480-512			
201W-6	1901434	155-218	6/6/97	MSGBVWM	<4
		254-274			
		405-425			
201W-6	1901434	155-218	6/9/97	PRODUCER	<4
		254-274			

Attachment 1. Perchlorate in Raw Groundwater - Monitoring and Production Wells (from Water Quality Authority Database)

		405-425			
201W-6	1901434	155-218	6/11/97	DHS	<4
		254-274			
		405-425			
Valley County Water District					
Morada	1900029	275-585	5/2/97	DHS	12
Morada	1900029	275-585	5/2/97	DHS	13
Lante	8000060	275-577	5/13/97	DHS	63
Lante	8000060	275-577	5/13/97	DHS	62
Lante	8000060	275-577	6/3/97	DHS	61
Lante 1/	8000060	275-577	6/3/97	MSGBVWM	65
Lante	8000060	275-577	6/6/97	DHS	64
Lante	8000060	275-577	6/6/97	DHS	66
Lante	8000060	275-577	6/6/97	DHS	67
Joanbridge East	1900032	300-586	5/29/97	DHS	<4
Joanbridge West	1902356	300-584	5/29/97	DHS	<4
Maine East	1900027	250-580	5/29/97	DHS	<4
Maine West	1900028	250-580	5/29/97	DHS	<4
Footnotes:					
1/ Split sample with DHS					
2/ On June 13, 1997 producer turned off the well due to high concentrations of Perchlorate.					
DHS: California Department of Health Services					
MSGBVWM: Main San Gabriel Basin Watermaster					
PPB: Parts per billion					

Attachment 2

PERCHLORATE IN PRODUCTION WELLS WITH TREATMENT
(from Water Quality Authority Database)

Attachment 2. Perchlorate in Production Wells with Treatment (from Water Quality Authority Database)

SAMPLING_SOURCE	RECORDATION_NUMBER	SAMPLING_DATE	SAMPLE_TYPE	RESULTS (PPB)
California Domestic Water Company				
Air Stripper #2 Effluent	1910199	6/9/97	Post Air Stripping	ND
Bassell Reservoir Effluent	1910199	6/9/97	Blend DW/chlorinated	ND
Bassell Reservoir Influent	1910199	6/9/97	Blend	ND
Well 3, 5A, 6 Blend	1910199	6/9/97	Blend	ND
Covina Irrigating Company				
Baldwin Park Reservoir	1910128	6/3/97	Chlorinated	ND
La Puente Valley County Water District				
3	1910060	6/10/97	Post Air Stripping	72
Miller Brewing Company				
Combined Effluent of GAC Vessels		6/5/97	GAC Effluent	ND
GAC Vessel #4		6/5/97	GAC Effluent	ND
San Gabriel Valley Water Company				
Plant B4 Reservoir	1910039	6/3/97	Blend	ND
Plant B4 Reservoir	1910039	6/3/97	Blend/chlorinated	ND
Reservoir B12	1910039	6/9/97	Blend DW/chlorinated	ND
Reservoir B5 large	1910039	6/9/97	Blend/chlorinated	5
Reservoir B5 small	1910039	6/9/97	Blend/chlorinated	4
Reservoir B6	1910039	6/3/97	Blend DW/chlorinated	24
Reservoir B6	1910039	6/9/97	Blend/chlorinated	29
B11B	1910039	6/9/97	Post Air Stripping	ND
B6C	1910039	6/3/97	Post Air Stripping	77
B6C	1910039	6/9/97	Post Air Stripping	72
B6D	1910039	6/3/97	Post Air Stripping	ND
B6D	1910039	6/9/97	Post Air Stripping	ND
Well B6C & B6D Effluent	1910039	6/3/97	Blend	42
Well B6C & B6D Effluent	1910039	6/9/97	Blend	38
B7C	1910039	6/9/97	Post Air Stripping	ND

Attachment 2. Perchlorate in Production Wells with Treatment (from Water Quality Authority Database)

Suburban Water Systems				
Bartola Well 201W-4	1910174	6/9/97	Chlorinated	ND
Bartola Well 201W-6	1910174	6/11/97	Chlorinated	ND
Bartola Wells Blend	1910174	6/11/97	Blend/chlorinated	ND
Big Dalton GAC comb.	1910205	5/29/97	GAC Effluent	38.7
Big Dalton GAC comb.	1910205	6/3/97	Chlorinated	5
Big Dalton GAC comb.	1910205	6/6/97	Chlorinated	33
Big Dalton GAC comb.	1910205	6/11/97	Chlorinated	25
Big Dalton GAC #2 1/2	1910205	5/29/97	GAC Effluent	41 6
Big Dalton GAC #2 1/4	1910205	5/29/97	GAC Effluent	41.3
Big Dalton GAC #2 3/4	1910205	5/29/97	GAC Effluent	42
Big Dalton GAC #3 1/2	1910205	6/3/97	GAC Effluent	25
Big Dalton GAC #3 1/2	1910205	6/6/97	GAC Effluent	32
Big Dalton GAC #3 1/2	1910205	6/11/97	GAC Effluent	35
Big Dalton GAC #3 1/4	1910205	6/3/97	GAC Effluent	40
Big Dalton GAC #3 1/4	1910205	6/6/97	GAC Effluent	36
Big Dalton GAC #3 1/4	1910205	6/11/97	GAC Effluent	36
Big Dalton GAC #3 3/4	1910205	6/3/97	GAC Effluent	5
Big Dalton GAC #3 3/4	1910205	6/6/97	GAC Effluent	19
Big Dalton GAC #3 3/4	1910205	6/11/97	GAC Effluent	31
CA Reservoir 121 R1	1910205	5/29/97	Chlorinated	12.2
CA Reservoir 121 R1	1910205	6/3/97	Chlorinated	5
CA Reservoir 121 R1	1910205	6/6/97	Chlorinated	7
CA Reservoir 121 R1	1910205	6/11/97	Chlorinated	9
Reservoir 125 R1	1910205	6/3/97	Chlorinated	ND
Joanbridge East Well (06E)	1910009	5/29/97	Chlorinated	ND
Lante Reservoir	1910009	5/29/97	Chlorinated	6.6
Lante Reservoir	1910009	6/3/97	Chlorinated	10
Lante Reservoir	1910009	6/6/97	Drinking Water	8
Lante Reservoir	1910009	6/6/97	Drinking Water	8
Lante Reservoir	1910009	6/6/97	Drinking Water	8
Lante Well	1910009	6/6/97	AOP Influent	64
Lante Well	1910009	6/6/97	AOP Effluent	64
Lante Well	1910009	6/6/97	GAC Effluent (primary vessel)	45
Lante Well	1910009	6/6/97	GAC Effluent (polishing vessel)	10
Lante Well	1910009	6/6/97	GAC Combined Effluent	17
Lante Well	1910009	6/6/97	GAC 1/2 depth polishing vessel	16

Attachment 2. Perchlorate in Production Wells with Treatment (from Water Quality Authority Database)

Lante Well	1910009	6/6/97	AOP Influent	66
Lante Well	1910009	6/6/97	AOP Effluent	66
Lante Well	1910009	6/6/97	GAC Effluent (primary vessel)	39
Lante Well	1910009	6/6/97	GAC Effluent (polishing vessel)	9
Lante Well	1910009	6/6/97	GAC Combined Effluent	17
Lante Well	1910009	6/6/97	GAC 1/2 depth polishing vessel	12
Lante Well	1910009	6/6/97	AOP Influent	67
Lante Well	1910009	6/6/97	AOP Effluent	68
Lante Well	1910009	6/6/97	GAC Effluent (primary vessel)	36
Lante Well	1910009	6/6/97	GAC Effluent (polishing vessel)	9
Lante Well	1910009	6/6/97	GAC Combined Effluent	16
Lante Well	1910009	6/6/97	GAC 1/2 depth polishing vessel	11
Lante Well GAC #2 1/2	1910009	6/3/97	GAC Effluent	14
Lante Well GAC #2 eff.	1910009	6/3/97	GAC Effluent	18
Lante Well GAC #1 eff.	1910009	6/3/97	GAC Effluent	7
Main GAC combined	1910009	5/29/97	GAC Effluent	ND
Main GAC #1 port 2?	1910009	5/29/97	GAC Effluent	ND
Main GAC #1 pot 3?	1910009	5/29/97	GAC Effluent	ND

Attachment 2. Perchlorate in Production Wells with Treatment (from Water Quality Authority Database)

COMMENTS
Blend of Wells 1, 5A, and 6 after air stripping.
Blend of Wells 2, 3, 5A, 6, and 8 after chlorination???
Blend of Wells 2, 3, 5A, and 6.
Blend of Wells 3, 5A, and 6 prior to air stripping.
Residual C12 was 0.52 mg/L
Prior to Chlorination.
After Chlorine Injection Point
Blend from Wells B11A, B11B, B9B, B7C, and B7E. Residual C12 was 0.75 mg/L.
Water from B5, well is transferred to B5 large for storage prior to distribution. Residual C12 was 0.62 mg/L.
Blend from Wells B5A, B5B, and B5C. Residual C12 was 0.59 mg/L.
Residual C12 was 0.52 mg/L.
Blend from Wells B6C,and B6D. Residual C12 was 0.51 mg/L.
After Air Stripping
After Air Stripping
After Air Stripping
After Air Stripping
Combined effluent after air stripping.
Combined effluent after air stripping.

Attachment 2. Perchlorate in Production Wells with Treatment (from Water Quality Authority Database)

Residual C12 was 0.09 mg/L.
Residual C12 was 0.08 mg/L.
Sample taken at the Chlorination Building. Residual C12 was 1.09 mg/L.
GAC vessels 1 & 2 combined effluent blend.
Combined effluent from GAC Vessels #3 & 4. Residual C12 was 1.49 mg/L.
Combined effluent from GAC Vessels #3 & 4. Residual C12 was 0.92 mg/L.
Combined effluent from GAC Vessels #3 & 4. Residual C12 was 0.84? mg/L.
Sampled at 1/2 depth, prior to breakthrough.
Sampled at 1/4 depth, prior to breakthrough.
Sampled at 3/4 depth, prior to breakthrough.
Sampled at 1/2 depth, 5th day of filter run
Sampled at 1/2 depth, 7th day of filter run
Sampled at 1/2 depth, 12th day of filter run
Sampled at 1/4 depth, 5th day of filter run
Sampled at 1/4 depth, 7th day of filter run
Sampled at 1/4 depth, 12th day of filter run
Sampled at 3/4 depth, 5th day of filter run
Sampled at 3/4 depth, 7th day of filter run
Sampled at 3/4 depth, 12th day of filter run
Blend prior to distribution. Residual C12 was 0.63 mg/L.
Blend from Big Dalton, 139W-2, 139W-4, 139W-5, and 139W-6. Residual C12 was 0.7 mg/L.
Blend from Big Dalton, 139W-2, 139W-4, 139W-5, and 139W-6. Residual C12 was 0.94 mg/L.
Blend from Big Dalton, 139W-2, 139W-4, 139W-5, and 139W-6. Residual C12 was 0.92 mg/L.
Residual C12 was 0.70? mg/L.
Residual C12 was 0.13 mg/L.
Residual C12 was 0.11 mg/L.
Post AOP (secondary monohydrogen peroxide feed rate); sampled at 11:06am.
Post AOP (secondary monohydrogen peroxide feed rate); sampled at 11:46am.
Post AOP (secondary monohydrogen peroxide feed rate); sampled at 12:09pm.
AOP influent - Lante Res.; sampled at 10:40am.
AOP effluent (preliminary monohydrogen peroxide feed rate); sampled at 10:50am.
GAC cross-over between primary and secondary vessels, sampled at 10:51am.
GAC effluent of polishing vessel; sampled at 10:59am.
Combined effluent of both GAC vessel union (note: flow in one vessel train is higher than the other due to carbon placement and stainer differences?); sampled at 11:01am
Post AOP (final monohydrogen peroxide feed rate); sampled at 10:56am.

Attachment 2. Perchlorate in Production Wells with Treatment (from Water Quality Authority Database)

AOP Influent - Lante Raw; sampled at 11:30am
AOP Effluent (preliminary monohydrogen peroxide feed rate); sampled at 11:29am.
GAC cross-over between primary and secondary vessels, sampled at 11:32am.
GAC effluent of polishing vessel; sampled at 11:38am.
Combined effluent of both GAC vessel union (note: flow in one vessel train is higher than the other due to carbon placement and stainer differences?); sampled at 11:42am
Post AOP (final monohydrogen peroxide feed rate); sampled at 11:15am.
AOP Influent - Lante Raw; sampled at 12:10pm
AOP Effluent (preliminary monohydrogen peroxide feed rate); sampled at 12:12pm.
GAC cross-over between primary and secondary vessels, sampled at 11:57am.
GAC effluent of polishing vessel; sampled at 12:03am.
Combined effluent of both GAC vessel union (note: flow in one vessel train is higher than the other due to carbon placement and stainer differences?); sampled at 12:09pm
Post AOP (final monohydrogen peroxide feed rate); sampled at 12:09pm.
Sampled at 1/2 depth.
GAC vessels 1 & 2 combined effluent blend.
Sampled at 1/2 depth.
Sampled at 3/4 depth.

Attachment 3

LITERATURE SEARCH FROM THE SUTTER/CHS HEALTH RESOURCE CENTER LIBRARY

Sutter/CHS Health Resource Center

Library

Name: Maryann Castaneda Date: 1-27-97

This is the information you requested on:

Attached is all we could come up with on your subject.
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Sacramento, California 95816

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USER:
ts (MH) human
PROG:
SS (3) PSTG (23)

SS 4 /C?
USER:
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PROG:

1
AU  - Trip MD ; Duren DR ; Wiersinga WM
TI  - Two cases of amiodarone-induced thyrotoxicosis successfully
      treated with a short course of antithyroid drugs while amiodarone
      was continued.
SI  - TOXBIB/95/033710
SO  - Br Heart J; VOL 72, ISS 3, 1994, P266-8
AB  - Two patients with amiodarone-induced thyrotoxicosis were treated
      successfully with potassium perchlorate and carbimazole while
      treatment with amiodarone was continued. These antithyroid drugs
      were stopped after the patients had became clinically and
      biochemically euthyroid. During follow up, when treatment with
      amiodarone continued, thyrotoxicosis did not recur.
      Amiodarone-induced thyrotoxicosis seems to be a transient
      condition that can be treated successfully with a short course of
      antithyroid drugs without stopping amiodarone treatment.

2
AU  - Laure P ; Stierle F
TI  - Methaemoglobinaemia: an unusual case report [letter]
SI  - TOXBIB/93/253187
SO  - Intensive Care Med; VOL 19, ISS 2, 1993, P124

3
AU  - van Dam EW ; Prummel MF ; Wiersinga WM ; Nikkels RE
TI  - Treatment of amiodarone-induced hypothyroidism with potassium
      perchlorate.
SI  - TOXBIB/93/189042
SO  - Neth J Med; VOL 42, ISS 1-2, 1993, P21-4
AB  - The antiarrhythmic drug, amiodarone, induces thyroid dysfunction,
      which is potentially dangerous in cardiac patients. After
      discontinuation of the drug it takes several months before
      euthyroidism is restored. The potent antithyroid drug, potassium
      perchlorate (KClO4), is used successfully to treat
      amiodarone-induced thyrotoxicosis, but it is less well known as
      potential treatment in amiodarone-induced hypothyroidism. In this
      case report we describe the successful use of two courses of
      KClO4 treatment in a cardiac patient with severe
      amiodarone-induced hypothyroidism. The mechanisms responsible for
      the amiodarone-induced hypothyroidism and rationale for the use
      of KClO4 in this condition are discussed.
CONTINUE PRINTING? (YES/NO)

USER:
y
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4
AU - Spiller DG ; Tidd DM
TI - Abrogation of c-MYC protein degradation in human lymphocyte lysates by prior precipitation with perchloric acid.
SI - TOXBIB/92/259982
SO - J Immunol Methods; VOL 149, ISS 1, 1992, P29-35
AB - Conventional lysis buffers, though containing cocktails of protease inhibitors, did not prevent the degradation of c-MYC recombinant protein added immediately prior to lysis to cell pellets from human mixed lymphocyte cultures. Treatment of the cells with 4.2% perchloric acid, however, prevented protein degradation and facilitated the detection of c-MYC protein by Western blotting even in unstimulated lymphocytes, where previously it had been reported to be undetectable or barely detectable using this technique. PHA stimulation of lymphocytes induced an approximately six fold increase in measured c-MYC protein within 5 h if cell extracts were prepared using perchloric acid precipitation. However, using conventional lysis buffer the proto-oncogene protein was undetectable until 48-72 h after mitogen addition. Pretreatment with perchloric acid may be useful for Western blotting analysis of protein in other systems where it may be desirable to dispense with the use of toxic protease inhibitors or where these may be incompletely effective.

5
AU - Reichert LJ ; de Rooy HA
TI - Treatment of amiodarone induced hyperthyroidism with potassium perchlorate and methimazole during amiodarone treatment.
SI - TOXBIB/89/336049
SO - BMJ; VOL 298, ISS 6687, 1989, P1547-8
AB - To exploit the antiarrhythmic effect of amiodarone when patients develop the side effect of thyrotoxicosis three patients with hyperthyroidism induced by amiodarone were given simultaneously 1 g potassium perchlorate a day for 40 days and a starting dose of 40 mg methimazole a day while they continued to take amiodarone. As hyperthyroidism might have recurred after potassium perchlorate treatment was stopped the dose of methimazole was not reduced until biochemical hypothyroidism (raised thyroid stimulating hormone concentrations) was achieved. The patients became euthyroid (free triiodothyronine concentration returned to normal values) in two to five weeks and hypothyroid in 10 to 14 weeks. One patient became euthyroid while taking 5 mg methimazole a day and 600 mg amiodarone weekly; the two others required substitution treatment with thyroxine sodium while taking 5 mg methimazole or 50 mg propylthiouracil (because of an allergic reaction to methimazole) and 2100 or 1400 mg amiodarone weekly. Hyperthyroidism induced by amiodarone may be treated with potassium perchlorate and methimazole given simultaneously while treatment with amiodarone is continued.
CONTINUE PRINTING? (YES/NO)

USER:
Y
DG:

6
AU - Dal Fabbro S ; Dalle Mule I ; Bridda A
TI - More on KClO(4) and amiodarone associated thyrotoxicosis [letter]
SI - TOXBIB/90/[illegible]

[redacted]

)G:

)U - Dal Fabbro S ; Dalle Mule I ; Bridda A
TI - More on KC10(4) and amiodarone associated thyrotoxicosis [letter]
)I - TOXBIB/89/124204
)O - J Endocrinol Invest; VOL 11, ISS 9, 1988, P691-2

)U - Bonnyns M ; Bourdoux P
TI - Delayed control of iodine-induced thyrotoxicosis with a thionamide after KClO4 withdrawal [letter]
)I - TOXBIB/89/035293
)O - J Endocrinol Invest; VOL 11, ISS 5, 1988, P393

AU - Newnham HH ; Topliss DJ ; Le Grand BA ; Chosich N ; Harper RW ; Stockigt JR
TI - Amiodarone-induced hyperthyroidism: assessment of the predictive value of biochemical testing and response to combined therapy using propylthiouracil and potassium perchlorate.
SI - TOXBIB/88/280687
SO - Aust N Z J Med; VOL 18, ISS 1, 1988, P37-44
AB - In order to assess the value of thyroid function testing during amiodarone therapy, we reviewed all available tests in 128 patients treated with this drug. Nine patients (7.0%) developed biochemical hyperthyroidism with elevation of both free thyroxine index (FT4I) and free triiodothyronine index (FT3I) and marked suppression of serum thyroid stimulating hormone (TSH) after 1-46 months of therapy; six of these nine patients had clear clinical evidence of thyroid overactivity. Where serial tests were available before development of hyperthyroidism, this complication developed suddenly, despite previously stable normal indices of thyroid function, and could not be predicted by currently-available biochemical tests such as T4, T3, sensitive TSH, thyroglobulin or sex hormone binding globulin (SHBG) assays. Clinical features such as unexplained weight loss, proximal myopathy, exacerbation of arrhythmia, or heat intolerance appear to be the key to prompt diagnosis of this complication. Hyperthyroxinemia without T3 excess was found in 32.8% of patients without progression to true hyperthyroidism. Serum TSH remained detectable by sensitive assay in 17 out of 18 patients with amiodarone-induced euthyroid hyperthyroxinemia and was significantly higher than in patients with equivalent hyperthyroxinemia due to thyroxine therapy. Serial levels of SHBG were higher in patients with true hyperthyroidism than in those with euthyroid hyperthyroxinemia. The effect of combined treatment with propylthiouracil (800 mg/day) and potassium perchlorate (800 mg/day) was evaluated in five of the six clinically hyperthyroid patients. Biochemical euthyroidism was achieved after 7-19 weeks, a response slower than previously reported, indicating that this drug combination does not result uniformly in prompt resolution of amiodarone-induced hyperthyroidism.
CONTINUE PRINTING? (YES/NO)

:E?:

'S:

'OG:

- Tsukamoto S ; Muto T ; Chiba S ; Sudo T ; Shibuya Y ; Osnide S
- An improved perchloric acid method for determination and stability of human blood acetaldehyde.
- TOXBIB/88/133535
- Arukoru Kenkyuto Yakubutsu Ison; VOL 22, ISS 3, 1987, P203-10

- Martino E ; Aghini-Lombardi F ; Mariotti S ; Lenziardi M ; Baschieri L ; Braverman LE ; Pinchera A
- Treatment of amiodarone associated thyrotoxicosis by simultaneous administration of potassium perchlorate and methimazole.
- TOXBIB/87/009636
- J Endocrinol Invest; VOL 9, ISS 3, 1986, P201-7
- Amiodarone iodine induced thyrotoxicosis occurs frequently in patients residing in areas of mild iodine deficiency and in patients with preexisting goiter. Drug therapy of the hyperthyroidism is often unsuccessful. Twenty-three patients with amiodarone induced thyrotoxicosis were either not treated, treated with 40 mg methimazole daily or with methimazole and 1 gm potassium perchlorate daily for up to 40 days and then with methimazole alone. Thyrotoxicosis was more likely to spontaneously remit in patients without goiter. Therapy with methimazole alone was unsuccessful in inducing euthyroidism in 5 patients with goiter. However, combined therapy with methimazole and potassium perchlorate rapidly alleviated hyperthyroidism in almost all patients with goiter. This drug combination is successful because perchlorate inhibits the active transport of iodine into the thyroid and methimazole blocks the intrathyroidal synthesis of thyroid hormones.

ONTINUE PRINTING? (YES/NO)

'SER:

'ROG:

- Martino E ; Mariotti S ; Aghini-Lombardi F ; Lenziardi M ; Morabito S ; Baschieri L ; Pinchera A ; Braverman L ; Safran M
- Short term administration of potassium perchlorate restores euthyroidism in amiodarone iodine-induced hypothyroidism.
- TOXBIB/87/008909
- J Clin Endocrinol Metab; VOL 63, ISS 5, 1986, P1233-6
- We studied the effect of potassium perchlorate (KC104) in patients with hypothyroidism due to amiodarone. The short term administration of KC104 to six such patients led to prompt restoration of euthyroidism, while the three untreated patients remained hypothyroid for 2-6 months. Since KC104 inhibits thyroid iodide transport, thereby blocking further entrance of iodide into the thyroid and decreasing intrathyroidal iodide content, amiodarone-associated hypothyroidism is probably secondary to the inhibitory effect of excess intrathyroidal iodine on thyroid hormone synthesis.

- Wenzel KW ; Lente JR
- Similar effects of thionamide drugs and perchlorate on

Human evidence

AU - Wenzel KW ; Lente JR
TI - Similar effects of thionamide drugs and perchlorate on thyroid-stimulating immunoglobulins in Graves' disease: evidence against an immunosuppressive action of thionamide drugs.
SI - TOXBIB/84/062173
SO - J Clin Endocrinol Metab; Vol 58, ISS 1, 1984, P62-9
AB - Previous studies have shown that serum titers of thyroid-specific antibodies such as thyroid-stimulating immunoglobulins (TSI), TSH-displacing antibodies (TDA), or microsomal antibodies (MAb) decrease in patients with Graves' disease during therapy with thionamide drugs (TD). In keeping with some in vitro results it was postulated that TD have an immunosuppressive action which may be partly responsible for the beneficial effects. To further elucidate this theory, we compared the changes in TSI during treatment with TD such as methimazole (MMI) and propylthiouracil (PTU) as well as with perchlorate (PC), an unrelated compound with a different mode of therapeutic action. Of 69 patients with hyperthyroidism due to Graves' disease, serum from 62 (90%) was positive for TSI, as measured by cAMP accumulation in a thyroid tissue culture assay. Six patients had to be excluded due to noncompliance. Of the remaining 56 patients, those 41 subjects (73%) with good control of the disease were followed up to 24 months during dose-adjusted antithyroid treatment. All patients with an uncomplicated course of treatment had a decline in the initially increased TSI values on either drug regimen. Five of 10 patients receiving PTU and 8 of 13 patients receiving MMI reached normal TSI levels; so did 11 of 18 patients receiving PC. There was no individual correlation between TSI decrease and drug dosages or the serum T4 and T3 levels. In all 3 groups, however, a decrease in mean T4 and T3 levels preceded the fall in TSI. By grouping the patients according to whether they had more than a 20% decrease in the initial TSI values after either 2 months or more than 4 months of treatment, it could be shown that the late responders had significantly higher T4 and T3 levels after 2 months of treatment. The similar patterns of change in TSI during treatment with TD and PC are strong evidence against an immunosuppressive effect of TD. If any direct interference occurs, a toxic effect on intrathyroidal lymphocytes by intrathyroidal drug accumulation could be the cause of the disappearance of TSI with both drug types. On the other hand, the data provide indirect evidence for the theory that the restoration of the euthyroid state is the cause of decreasing TSI levels and normalization of the immune regulation in many patients during treatment with antithyroid drugs.
CONTINUE PRINTING? (YES/NO)

USER:
y
PROG:

13
AU - Connell JM
TI - Long-term use of potassium perchlorate.
SI - TOXBIB/82/059910
SO - Postgrad Med J; Vol 57, ISS 670, 1981, P516-7
AB - A case of Graves' disease with potassium perchorate for 22 years without ill effect is described. Thyrotoxicosis recurred 4 weeks after the medication was withdrawn, suggesting that euthyroidism had been maintained by chronic use of the drug. As toxicity of perchlorate is probably dose related, it is suggested that long-term use of low dose perchlorate may be no more hazardous than alternative antithyroid therapy.

Postgraduate Medical Journal (August 1981) 57, 516-517

Long-term use of potassium perchlorate

J. M. C. CONNELL
M.B., M R.C.P.

Department of Medicine, Western Infirmary, Glasgow

Summary

A case of Graves' disease with potassium perchlorate for 22 years without ill effect is described. Thyrotoxicosis recurred 4 weeks after the medication was withdrawn, suggesting that euthyroidism had been maintained by chronic use of the drug. As toxicity of perchlorate is probably dose related, it is suggested that long-term use of low dose perchlorate may be no more hazardous than alternative antithyroid therapy.

Introduction

Potassium perchlorate was extensively used as an antithyroid agent in the late 1950s and early 1960s (Crooks and Wayne, 1960): by competitive inhibition of the trapping of iodide by the thyroid it was effective in reducing thyroid hormone production by the gland, and consequently in relieving symptoms of thyrotoxicosis (Godley and Stanbury, 1954). No evidence has been produced to suggest that it might influence the natural course of thyrotoxicosis. Following reports of toxicity, in particular of bone marrow hypoplasia (Barzilai and Sheinfeld, 1966) it fell into disfavour, and is now used mainly for investigative purposes. The author now reports a case of long-term use of potassium perchlorate.

Case report

A 72-year-old female was referred to the Thyroid Clinic in August 1980 with symptoms of thyrotoxicosis. She had undergone a partial thyroidectomy in another hospital in 1945 for thyrotoxicosis. In 1956, she was diagnosed as suffering from pernicious anaemia, and started on regular vitamin B_{12} therapy. In 1958, her thyrotoxicosis recurred both clinically and biochemically. She was rendered euthyroid with potassium perchlorate, one g/day by mouth for one month, and maintained thereafter on 200 mg/day, with good control of symptoms. She remained clinically euthyroid on this therapy without ill effect until May 1980, when her GP stopped the potassium perchlorate. Four weeks later she developed symptoms of thyrotoxicosis, including weight loss, heat intolerance and excessive sweating, and was referred to the Thyroid Clinic. Apart from pernicious anaemia affecting a maternal aunt, she gave no other history of note.

On examination she was clinically thyrotoxic, with warm moist palms and hyperkinetic movements. She had a tachycardia of 120 beats/min. A small diffuse goitre was palpable, with the left lobe being larger than the right; no bruit was audible. There was no ophthalmopathy. Other examination was unremarkable.

Initial thyroid function tests confirmed the clinical impression with a T_4 of 245 nmol/l (normal range 59–174), T_3 of 4·2 nmol/l (normal range 1·29–3·1) and a free thyroxine index of 77·4 (normal range 17·8–46·1) (Amersham radioimmunoassay kit). Thyroidal uptake of ^{123}I at 20 min after i.v. administration of the tracer was elevated at 9·7% of dose (normal range 2–8%); the precipitin test for thyroglobulin antibody was negative. A technetium scan of the thyroid showed a diffuse uptake of isotope, with the left lobe more active than the right. Haemoglobin was 11·3 g/dl with an MCV of 88 fl; WBC was $5·3 \times 10^9$/l, with normal film appearances; platelet count was normal at 194×10^9/l.

A diagnosis of Graves' disease seemed reasonable in view of her history of pernicious anaemia, and the diffuse thyroid scan appearance. In view of her age and recurrent nature of her illness, she was treated with radioactive iodine (^{131}I) by mouth, and is now clinically euthyroid.

Comment

This appears to be a unique case with maintenance of euthyroidism by the use of potassium perchlorate over a period of 22 years. The temporal relationship between withdrawal of perchlorate and recurrence of thyrotoxicosis suggests that perchlorate was responsible for the maintenance of euthyroidism, by continued chronic intrathyroidal iodine depletion. With the withdrawal of perchlorate, unblocked excessive iodide trapping was able to occur, leading to excessive thyroid hormone production; the

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situation is analogous to the cases of thyrotoxicosis 'unmasked' in populations by the introduction of dietary iodine supplementation (Connolly, Vidor and Stewart, 1970).

This patient received potassium perchlorate for 22 years without any untoward effects. The reports of adverse reactions to potassium perchlorate suggested that these effects of the drug were dose related (Morgans and Trotter, 1960), and it may be that low dose perchlorate (200 mg) is no more toxic than the current generation of thiourylene antithyroid drugs (Barzilai and Sheinfeld, 1966).

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Similar Effects of Thionamide Drugs and Perchlorate on Thyroid-Stimulating Immunoglobulins in Graves' Disease: Evidence against an Immunosuppressive Action of Thionamide Drugs

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ABSTRACT. Previous studies have shown that serum titers of thyroid-specific antibodies such as thyroid-stimulating immunoglobulins (TSI), TSH-displacing antibodies (TDA), or microsomal antibodies (MAb) decrease in patients with Graves' disease during therapy with thionamide drugs (TD). In keeping with some *in vitro* results it was postulated that TD have an immunosuppressive action which may be partly responsible for the beneficial effects. To further elucidate this theory, we compared the changes in TSI during treatment with TD such as methimazole (MMI) and propylthiouracil (PTU) as well as with perchlorate (PC), an unrelated compound with a different mode of therapeutic action.

Of 69 patients with hyperthyroidism due to Graves' disease, serum from 62 (90%) was positive for TSI, as measured by cAMP accumulation in a thyroid tissue culture assay. Six patients had to be excluded due to noncompliance. Of the remaining 56 patients, those 41 subjects (73%) with good control of the disease were followed up to 24 months during dose-adjusted antithyroid treatment. All patients with an uncomplicated course of treatment had a decline in the initially increased TSI values on either drug regimen. Five of 10 patients receiving PTU and 8 of 13 patients receiving MMI reached normal TSI levels; so did 11 of 18 patients receiving PC. There was no individual correlation between TSI decrease and drug dosages or the serum T_4 and T_3 levels. In all 3 groups, however, a decrease in mean T_4 and T_3 levels preceded the fall in TSI. By grouping the patients according to whether they had more than a 20% decrease in the initial TSI values after either 2 months or more than 4 months of treatment, it could be shown that the late responders had significantly higher T_4 and T_3 levels after 2 months of treatment.

The similar patterns of change in TSI during treatment with TD and PC are strong evidence against an immunosuppressive effect of TD. If any direct interference occurs, a toxic effect on intrathyroidal lymphocytes by intrathyroidal drug accumulation could be the cause of the disappearance of TSI with both drug types. On the other hand, the data provide indirect evidence for the theory that the restoration of the euthyroid state is the cause of decreasing TSI levels and normalization of the immune regulation in many patients during treatment with antithyroid drugs. (*J Clin Endocrinol Metab* 58: 62, 1984)

IN 1969, Pinchera *et al.* (1) reported a gradual decrease in LATS activity in the sera of Graves' patients who were treated with antithyroid drugs. In recent years, further longitudinal studies have also shown that TSH-displacing antibodies (TDA) (2–9), thyroid-stimulating immunoglobulins (TSI) (4, 9–11), and microsomal antibodies (MAb) (5, 12) often decline or disappear during treatment with thionamide drugs such as methimazole (MMI), carbimazole (CBI), or propylthiouracil (PTU). It was, therefore, proposed that thionamide drugs had an immunosuppressive effect upon antibody production (5, 8, 10). Some *in vitro* experiments supported this view (5, 13, 14). To examine this hypothesis, we studied the course of serum TSI levels during treatment with PTU, MMI, or perchlorate (PC) in similar groups of Graves' hyperthyroid patients (15). Unexpectedly, TSI activity in serum did not persist during therapy with PC, but showed a decline similar to that occurring during thionamide medication. To rule out uncertainty due to the small numbers of patients or the short duration of the study, we extended the survey to a total of 56 patients.

Although side effects of PC have not proven to be a major problem (16), this drug has not been frequently used for the treatment of hyperthyroidism during the last 2 decades. Before commencing this investigation, therefore, we reviewed the literature concerning adverse reactions during PC treatment. It was evident, however, from 2 large series of 200 (17) and 180 (18) patients, that severe adverse reactions, such as agranulocytosis, were likely to occur only when large doses of more than 1000 mg PC were administered, while doses lower than 1000 mg PC had fewer side effects than thionamide drugs.

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Materials and Methods

TSI assay

TSI values were determined by measuring cAMP accumulation in the supernatnt of primary tissue cultures of human

thyroid cells from specimens obtained from euthyroid patients with diffuse goiters (19). The original method was improved by a modified cell purification method developed for the separation of lymphocytes from thyroid tissue (20). In brief, resected thyroid specimens were minced, incubated with Dispase II (Boehringer Mannheim, New York, NY), and passed through a tissue sieve. The filtrate was layered onto a three-step discontinuous Percoll gradient (density, 1.077, 1.061, and 1.030 g/ml, respectively). Thyroid cells appeared in band II, whereas debris was separated in the upper band, and blood cells were separated in the lower band. Using a modification of the original method (19), a suspension of 10^6 viable cells/well was precultured in Falcon multiwell tissue plates (Falcon Plastics, Los Angeles, CA) for 24 h at 37 C before the addition of 0.2 ml serum to each well. After incubating the thyrocyte cultures for another 24 h, 50 μl of the spontaneous supernatant were taken for measuring cAMP by RIA (Becton Dickinson, Mechelen, Belgium). TSI activity was calculated by comparing the cAMP response (pmol cAMP/ml) induced by a test serum with the response to a pooled normal human serum. The results were expressed as the percentage of the control values:

$$\text{TSI} = \frac{\text{cAMP of patient serum} \times 100}{\text{cAMP of control pool serum}}$$

Since 50 individual normal sera had an average value of 100 ± 20% (SD) in comparison with the control pool, TSI activity higher than 120% was considered as positive. This was in exact concordance with the results of others who used this method of calculating TSI activity (21).

The sensitivity of this system, tested with various doses of bovine TSH, was 10 μU TSH/ml. The intraassay coefficient of variation for 10 single measurements of different sera ranged from 4.4–8.4%. The interassay variation in 12 different estimations showed a CV of 6.8% with 2 strongly positive sera, but other sera had wide variations or even became negative. Although the authors of the original method found that varying numbers of Graves' patients were TSI positive with different thyroid preparations, probably due to different properties of the cell membranes, repeated estimations with 5 different thyroid specimens yielded a 100% prevalence of TSI in Graves' patients (22). Therefore, in this study all samples from each single individual were always estimated in 1 TSI assay.

Hormone concentrations in serum were determined by commercial RIA assay kits, using normal ranges established in this laboratory: T_4 by Quantimmune-T_4-RIA (Bio-Rad, Richmond, CA), normal range: 4, 5–12, 4 μg/dl; T_3 by T_3-RIAcid (Henning, West Berlin, West Germany), normal range: 50–220 μg/dl.

Patients

Of 69 patients with unequivocal hyperthyroid Graves' disease, 62 (90%) subjects had measurable TSI activity in serum. Six patients were excluded because of noncompliance with therapy. Thus the longitudinal study was performed with 56 patients. In 7 the hyperthyroidism was difficult to control. One patient receiving MMI and 5 receiving PC required varying drug doses; excess iodine intake was a possible cause in the latter. One patient did not respond to 60 mg MMI and could only be controlled by addition of lithium carbonate. The data from 8 patients (2 on PTU, 5 on MMI, 1 on PC) who developed a syndrome of goiter enlargement, persisting TSI, low T_4 and borderline elevated T_3 have been published previously (23). Since the purpose of this study was to examine the effect of PC on serum TSI levels, only data from the remaining 41 patients with good control during the therapy will be presented.

All patients were studied at the time of their first occurrence of hyperthyroidism, which had a duration between 2 months and, at most, 2 yr (no differences in the subgroups). The average ages of the patients in the 3 subgroups were not significantly different [PTU group, 38 ± 12 (±SD) yr; MMI group, 39 ± 11 yr; PC group, 35 ± 14 yr]. Thyroid scan showed a normal sized thyroid in 12 out of the 41 patients, while diffuse enlargement was present in 8 of 10 subjects on PTU, 9 of 13 patients on MMI, and 12 of 18 subjects on PC treatment. Graves' ophtalmopathy was present in 8 of 10 (PTU), 9 of 13 (MMI), and 14 of 18 (PC) subjects.

Antithyroid treatment

Drug doses were adjusted according to serum T_4 and T_3 levels; usually the dose was halved after T_3 had reached the normal range. Treatment was started with three doses per day. After reduction to daily doses of 10 mg MMI or lower and 50 mg PTU or lower, only one dose in the morning was recommended, while PC was administered in two or three doses per day. For adjustment of drug doses, serum T_4 and T_3 were determined every 3–4 weeks during the early phase of treatment and subsequently every 6–8 weeks or at longer intervals after control of the disease.

Patients were assigned randomly to the subgroups for medication. Ten patients were treated with PTU, starting with 300 mg/day; the dosage was reduced gradually to final doses ranging from 25–100 mg PTU/day. Thirteen patients took MMI with gradual reduction from 60 mg to final doses of 2.5 to 10 mg/day. Eighteen patients received PC, starting with 900 mg; this was adjusted to 40–120 mg PC/day. None received thyroid hormones. Occasionally in the early phase, propranolol in low doses (40–60 mg/d) was given. The 38 women in this study did not take oral contraceptives.

Statistical analysis was performed using Student's *t* test for paired comparisons and the Wilcoxon rank test.

Results

The changes in TSI titers during treatment are depicted in Figs. 1–3. Individual values are given because the scatter of early and late responders could not have been recognized by drawing a line of average values. The initial TSI levels were between 130–340% in all 3 groups, and there was a gradual decline in TSI in all patients. During MMI treatment (Fig. 1), 8 of 13 patients reached the normal range after 4, 5, 8, 9, 10, 11, 12, and 14 months of treatment, respectively. In 2 patients, TSI became positive again after 20 and 22 months. In 7 of 10 patients on PTU (Fig. 2), initially elevated TSI values rose further during the early phase of treatment. Although such a small group allows only tentative conclusions, the delayed response to PTU treatment probably resulted from the initial dose of PTU (300 mg/day). On the other hand,

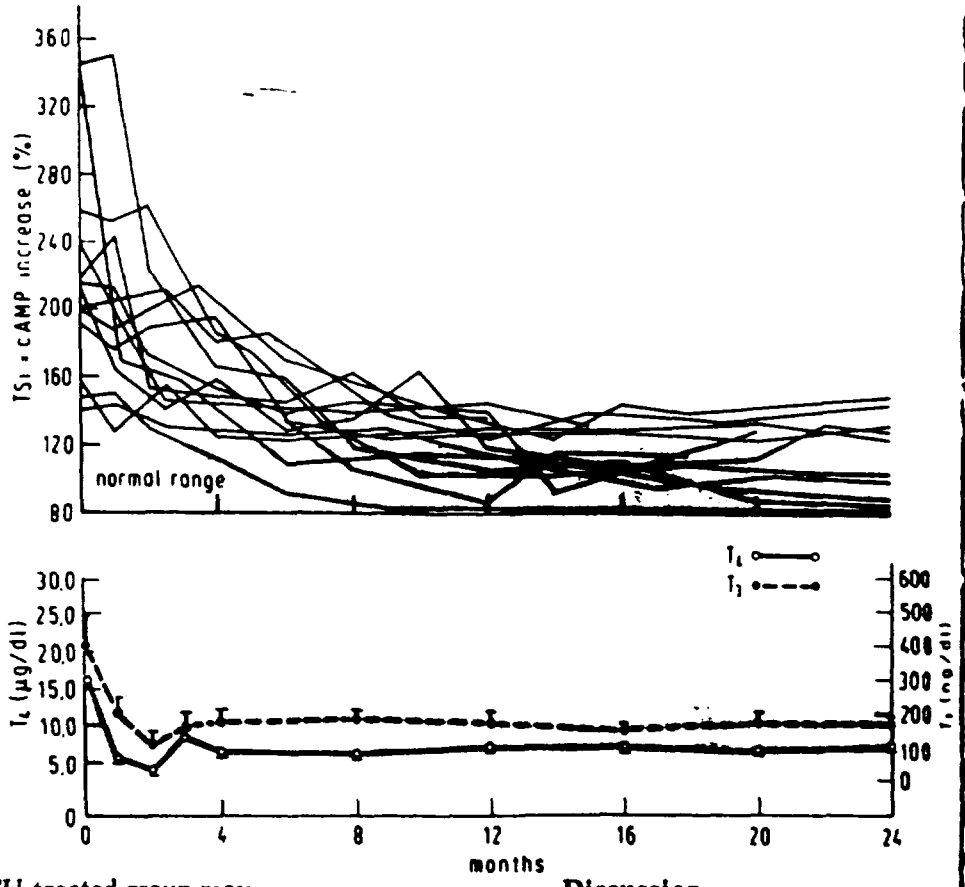

FIG. 1. Course of TSI in sera of 13 individual hyperthyroid Graves' patients during long term treatment with MMI (values *above the shaded area* are positive) and mean serum T_4 and T_3 values (the *vertical bars* represent the SD, and the *shaded area* depicts the normal range).

the severity of the disease in the PTU-treated group may have been more severe because the initial serum T_4 and T_3 levels and the proportion of patients with goiter and ophthalmopathy were higher in this group. After the initial rise, the TSI titers decreased, reaching the normal range after 5, 8, 9, 14, and 14 months, respectively. Eleven of 18 patients receiving PC (Fig. 3) also reached normal TSI values after 2, 2, 4, 8, 10, 10, 12, 12, 15, 18, and 20 months, respectively; 1 individual developed positive values after 20 months.

Statistical analysis of individual values did not show any correlation between TSI levels and serum T_4 and T_3 concentrations or drug dosages. Figures 1 and 3 show rapid normalization of the T_4 and T_3 levels, with subnormal T_4 levels after 2 months of MMI treatment and after 1–4 months of PC treatment. In PTU-treated patients (Fig. 2), the decreases in T_4 and T_3 were later and less pronounced because of the relatively low dose used, as mentioned above. The fall in individual and average values of T_4 and T_3 always preceded the decreases in TSI. Figure 4 shows that the group of late responders (>20% decrease in the initial TSI elevation after 4 months of treatment) had higher T_4 and T_3 concentrations during the first 2 months of treatment, even though the drug dosages were not different from those of early responders (>20% decrease in TSI within the first 2 months of medication).

Discussion

A gradual decrease in antithyroid antibodies occurs in the majority of patients with Graves' disease during treatment with thionamides (1–12). There has been increasing discussion as to whether this phenomenon is caused by immunosuppressive properties of thionamide drugs (5, 8, 10, 13, 14). *In vitro* studies of lymphocyte activity provide support for this assumption (3, 13, 14), but were not confirmed by others (24, 25). This longitudinal study was designed to obtain further information concerning the presumed immunosuppressive actions of MMI and PTU by comparing their TSI-suppressive behavior to that of the unrelated inorganic drug PC. It was striking, however, that the decreases in TSI activity in serum were similar during treatment with PC and during treatment with thionamide. These findings have implications for the understanding of the interaction between antithyroid drugs and autoimmune phenomena in Graves' disease. Several possible mechanisms have to be discussed.

The decline in TSI activity in serum could reflect remission of the underlying immunological disturbance (12). This theory could explain the persistence of TSI in a minority of patients treated with either drug and its disappearance in the others. If changes in TSI reflect the natural course of the disease, the decrease in TSI should

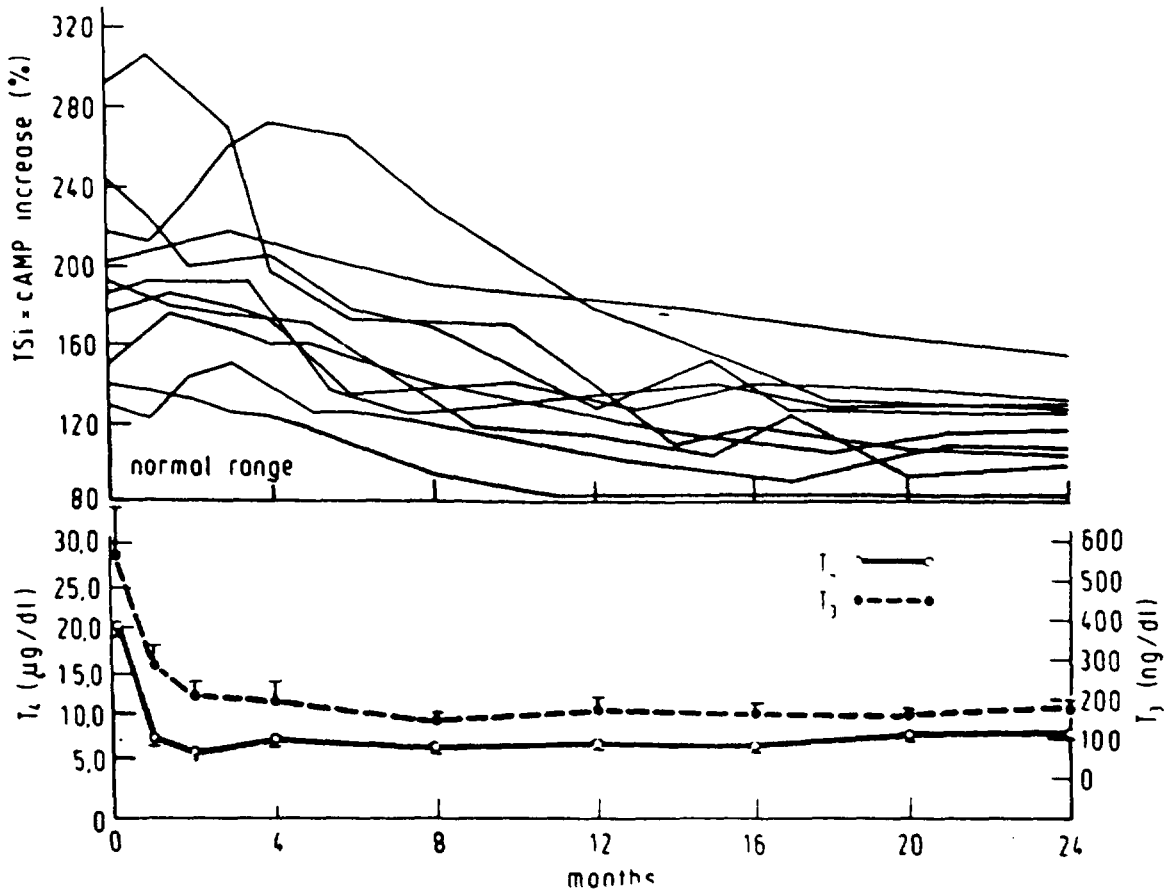

FIG. 2. Course of TSI in sera of 10 individual hyperthyroid Graves' patients during long term treatment with PTU (values *above the shaded area* are positive) and mean serum T_4 and T_3 values (the *vertical bars* represent the SD, and the *shaded area* depicts the normal range).

be distributed over varying time intervals during treatment with antithyroid drugs. However, it is obvious from this and other studies that the onset of TSI decline is similar in most responding patients; a pronounced fall in TSI and TDA usually occurred during the first 2 months (1, 3, 5, 7, 8, 10). A more rapid decline in TSI during the first 2 months of therapy was related to lower serum T_4 and T_3 in the early phase of treatment, indicating a more complete blocking effect on thyroid hormone synthesis. Furthermore, a decrease in TSI levels did not occur in 16 patients during 2 months of propranolol treatment (3).

A general immunosuppressive effect of antithyroid drugs is unlikely for several reasons. It would not explain why antithyroid antibodies persist in some patients in spite of treatment; moreover, a low dose regimen (1, 4, 8, 15) achieved virtually the same results as larger doses (3, 5, 6, 8, 10, 23). Since there have been no suggestions that PC has immunosuppressive activity, the findings during PC treatment do not support the existence of an immunological action of antithyroid drugs as they are used clinically. In addition, studies on mice immunized with sheep red blood cells provided no support that MMI had any action on the antibody-forming system (1). More recently, *in vitro* experiments have shown that MMI and PTU are capable of reducing the secretion of total immunoglobulin and MAb (5) or thyroglobulin antibodies (14) by pokeweed mitogen-stimulated lymphocytes from patients with Hashimoto's thyroiditis. However, whether this effect occurs *in vivo* remains to be established, because serum concentrations of MMI (26–28) or PTU (29–31) are at least 10 times lower than the effective *in vitro* concentrations, *i.e.* 10^{-5} *vs.* 10^{-4} M (5, 13, 14, 24, 25).

McGregor *et al.* (5) postulated that the beneficial effects of thionamide drugs in Graves' disease depend on their immunosuppressive action upon lymphocytes accumulated in the thyroid gland. This concept is based on the findings of McLachlan *et al.* (32) that lymphocytes from the thyroid glands of Hashimoto patients were capable of synthesizing MAb in larger amounts than peripheral lymphocytes. Such specific production by intrathyroidal lymphocytes was recently shown by the same authors for thyroglobulin antibodies from Hashimoto's (33) and Graves' thyroid preparations (34). Thionamide concentrations in the thyroid are about 10-

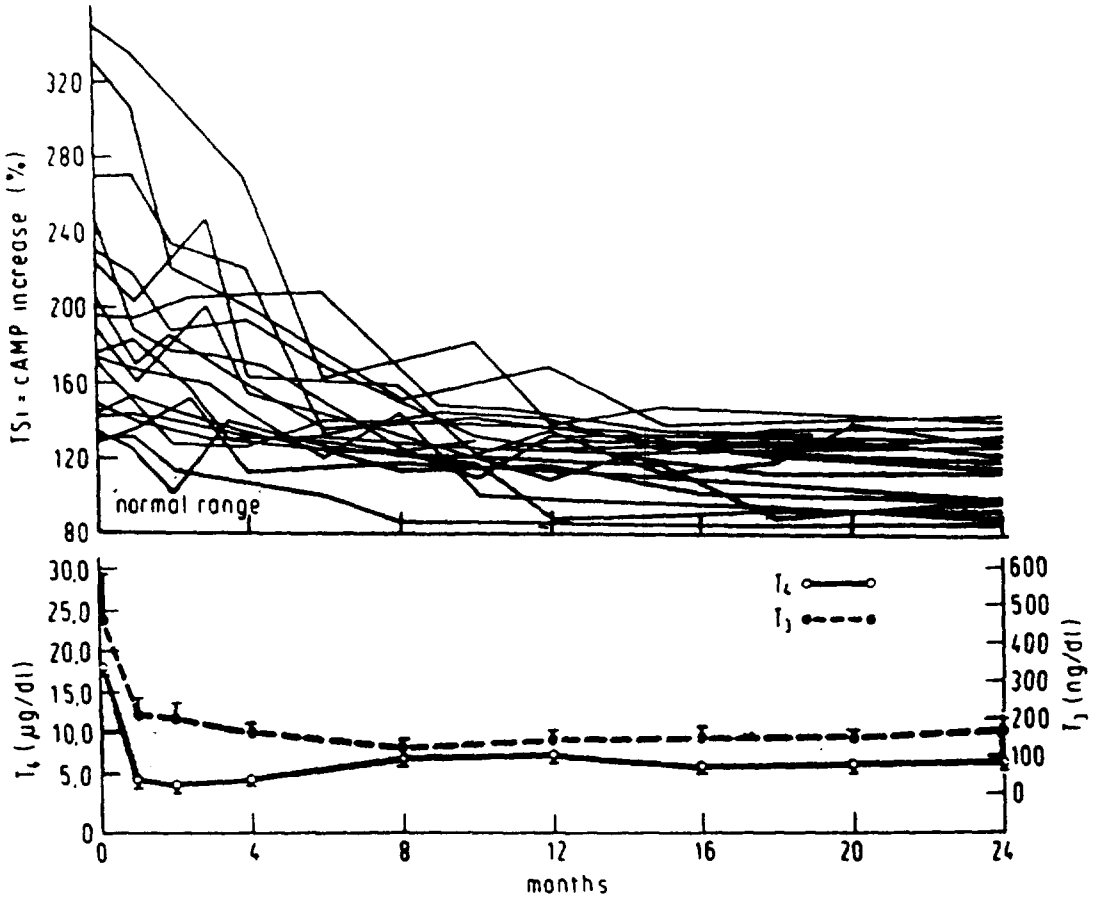

FIG. 3. Course of TSI in sera of 18 individual hyperthyroid Graves' patients during long term treatment with PC (values *above the shaded area* are positive) and mean T_4 and T_3 values (the *vertical bars* represent the SD, and the *shaded area* depicts the normal range).

fold higher than those in serum (35, 36), close to the concentrations shown to be immunosuppressive *in vitro* (5, 14). The possibility of inhibition of lymphocytes in the thyroid by thionamides is supported by the observation of reduced lymphocytic infiltration of the thyroid during CBI treatment (37) and by a fall in thyroid-specific TDA (with persistence of gastric parietal cell antibodies) during treatment with CBI in Graves' disease (38).

The concept of intrathyroidal immunosuppression of lymphocytes by thionamide drugs would have been supported by an opposite result of this study, *i.e.* persistence of elevated TSI levels during therapy with PC. Presuming that intrathyroidal lymphocytes are responsible for thyroid antibody production, one could speculate that selective accumulation of PC in the gland has toxic effects on these lymphocytes, and that thionamides have the same action. Unfortunately, no data are available concerning PC concentrations in the thyroid gland. Indirect evidence may be derived from the results of PC discharge tests, where PC is capable of displacing up to 96.6% of radioactive iodine during CBI treatment (39). Considering that at least one PC ion is necessary for displacing one iodine ion, and that the molar weights of PC and iodine are similar, one may calculate from the iodine content of normal thyroids in Germany (40, 41) a concentration of about 40 mg PC/g thyroid ($\sim 0.5 \times 10^{-4}$ M). This concentration strikingly corresponds to preliminary *in vitro* results where PC concentrations lower than 5×10^{-2} M did not affect the viability of lymphocytes as judged by trypan blue exclusion. On the other hand, thionamides, whose maximal intrathyroidal concentrations are 10^{-4} M (35, 36), would not achieve concentrations sufficient to produce a toxic action upon lymphocytes *in vitro* that require 10^{-3} M (5, 14).

Moreover, PC as well as MMI and PTU must be located mainly in the follicular cells. Those compounds are, therefore, remote from the clusters of lymphocytes and interaction with them seems somewhat unlikely. Furthermore, accumulation of MMI in intrathyroidal lymphocytes was recently excluded (42). Taken together, these results do not support the theory that antithyroid drugs have an immunosuppressive action on intrathyroidal lymphocytes; if there is any such action, it would be a toxic one depending on local drug concentration.

Another mechanism could be that both drug types

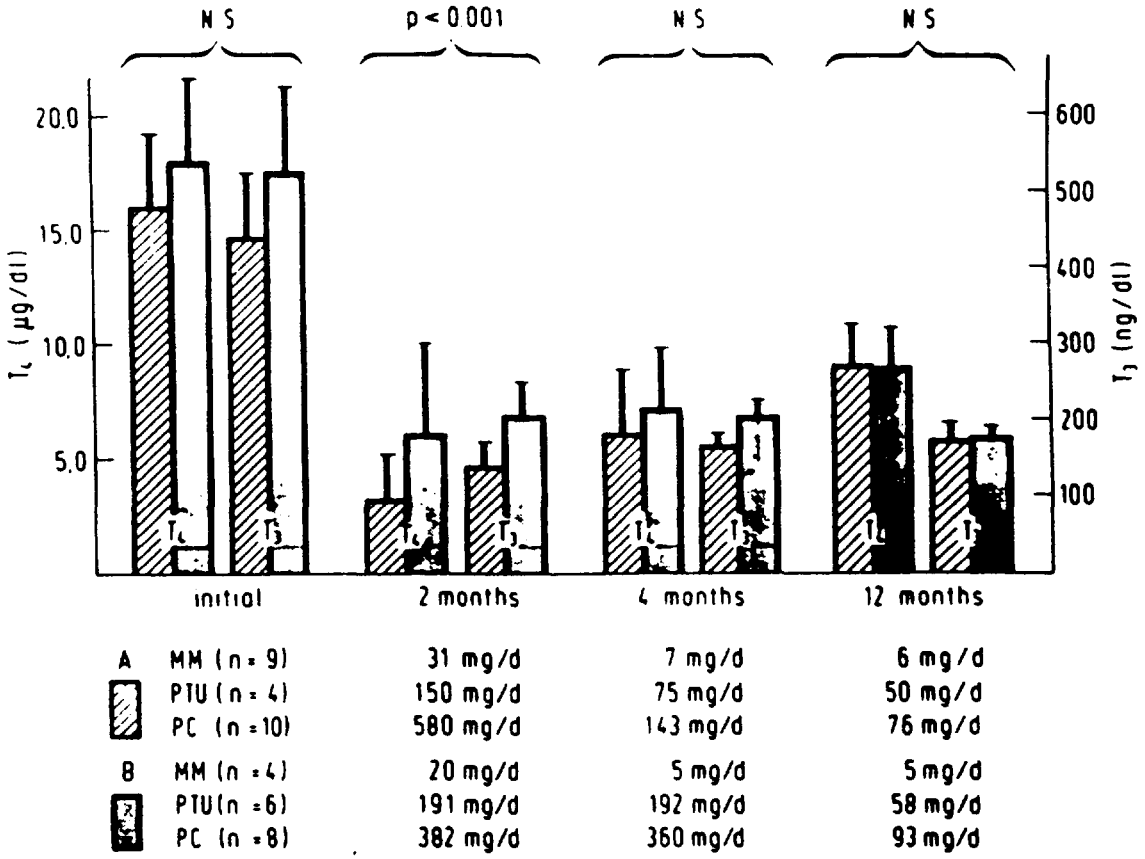

FIG. 4. Mean serum T_4 and T_3 levels during antithyroid treatment with MMI, PTU, or PC. Group A represents early responders, with a more than 20% fall in serum TSI values within 2 months of treatment; group B represents late responders, with a more than 20% fall in TSI only after 4 months of therapy. Serum T_4 and T_3 levels in group A were significantly lower after 2 months of treatment, whereas drug dosages at 2, 4, and 12 months after the commencement of therapy were similar in groups A and B.

cover antigenic sites, thus reducing antigen availability. This possibility may be supported by the findings that PTU and PC both inhibited the binding of TSH to bovine thyroid membranes at concentrations of 10^{-5}–10^{-3} M, whereas lower concentrations had stimulatory effects (43).

Finally, and most likely, the decline in TSI activity in serum could be due to the restoration of immunological regulation as the patient becomes euthyroid again (44). Current understanding of autoimmune responses assumes that humural antibodies result from a disturbance in lymphocyte immunoregulation (45). Animal experiments have shown that hyperthyroidism itself also results in diminished T suppressor lymphocyte function, thus tending to be a self-perpetuating factor (46). When the tendency for normalization of the abnormal suppressor function after treatment (47–49) is taken into account, it would appear that the hyperthyroid state itself alters immune function. Abnormal lymphocyte responses in patients with Graves' hyperthyroidism returned to normal when the patients became euthyroid (50), although some reports were not confirmatory (51, 52). The evaluation of lymphocyte subsets in hyperthyroid Graves' patients revealed a decrease in T suppressor cells, which was partly abolished after achievement of euthyroidism by PTU treatment or radioiodine therapy (53). Using an automated cell sorter, a qualitative, but not quantitative, abnormality of T suppressor cells was found during hyper- or hypothyroid states in patients with autoimmune thyroid disorders (54). Diminished splenic T suppressor cell counts in hypothyroid rats were normalized by T_3; however, experimental hyperthyroidism did not cause a change, perhaps due to the very short duration (17 days) of T_3-induced hyperthyroidism (55). A recent report described an inverse correlation between T_4 lymphocytes and thyroid hormone levels in Graves' patients, in so far as hyperthyroid patients with or without antithyroid medication (presumably thionamides) had diminished counts, while euthyroid patients receiving antithyroid medication or in remission had normal counts. Although it is not yet clear whether T_4 lymphocytes have mainly cytotoxic or suppressor cell properties, these results support the view that thyroid hormone levels alter lymphocyte populations and properties (56).

The present study, of course, does not provide direct support for the possible influence of the hyperthyroid state on the immune responses, in part because nonresponders with respect to TSI decrease were also maintained in a euthyroid state. [A subgroup of eight patients with a distinct syndrome of low T_4 and high T_3 levels has been recorded separately (23).] However, it can be seen from Figs. 1-3 that in all three groups of thionamide or PC-treated patients, a decrease in the mean serum T_4 and T_3 levels preceded the fall in TSI; such were also the individual changes. Similar patterns were reported by others (2, 3, 10). Figure 4 demonstrates that patients with a more rapid decrease in TSI had concomitantly more pronounced decreases in serum T_4 and T_3.

In conclusion, the findings during PC treatment do not confirm the theory of either a general or an intrathyroidal immunosuppressive action of thionamide drugs, although an intrathyroidal toxic effect of both thionamides and PC cannot be completely excluded. While not proven, it seems most likely that restoration of the euthryoid state by the blocking effect on biosynthesis of thyroid hormones is the cause of the decreasing TSI levels during antithyroid drug therapy in many patients. In some patients, the defect in immunoregulation may be so severe that restoration of a euthyroid state makes no substantial difference in the TSI levels.

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Treatment of amiodarone associated thyrotoxicosis by simultaneous administration of potassium perchlorate and methimazole[1]

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ABSTRACT. Amiodarone iodine induced thyrotoxicosis occurs frequently in patients residing in areas of mild iodine deficiency and in patients with preexisting goiter. Drug therapy of the hyperthyroidism is often unsuccessful. Twenty-three patients with amiodarone induced thyrotoxicosis were either not treated, treated with 40 mg methimazole daily or with methimazole and 1 gm potassium perchlorate daily for up to 40 days and then with methimazole alone. Thyrotoxicosis was more likely to spontaneously remit in patients without goiter. Therapy with methimazole alone was unsuccessful in inducing euthyroidism in 5 patients with goiter. However, combined therapy with methimazole and potassium perchlorate rapidly alleviated hyperthyroidism in almost all patients with goiter. This drug combination is successful because perchlorate inhibits the active transport of iodine into the thyroid and methimazole blocks the intrathyroidal synthesis of thyroid hormones.

INTRODUCTION

Amiodarone, a benzofuranic derivative containing 37.5 mg of iodine per 100 mg of active drug, is widely used for the treatment of tachyarrhythmias and ischemic heart disease and has a prolonged half life of approximately 3-4 months (1-7). This drug inhibits the outer ring, 5'-deiodination, of iodothyronines resulting in an increase in serum T_4 concentrations, a decrease in serum T_3 concentrations and an increase in serum reverse T_3 concentrations (8-13). Furthermore, some patients treated with amiodarone develop clinical and biochemical evidence of iodine induced hyperthyroidism or hypothyroidism (3, 5, 6, 14-18). Amiodarone associated iodine induced thyrotoxicosis (AAT) is more frequently observed in areas of mild iodine deficiency (Continental Europe) than in areas of sufficient iodine intake (USA and UK) (18). AAT is often a serious complication since it develops in patients with cardiac disorders and because its treatment has thus far been disappointing.

This represents a difficult clinical challenge. Thionamide drug therapy is generally less effective in this condition (19-23), the frequently observed low thyroid radioiodine uptake prevents the use of radioactive iodine (^{131}I) and thyroidectomy is often too dangerous to be considered in cardiac patients with uncontrolled thyrotoxicosis. Recently Wimpfheimer et al. (20) reported that the administration of prednisone with carbimazole did control the hyperthyroidism in two patients with AAT. The present study describes our experience with various antithyroid drug regimens in a large number of patients with AAT and suggests that the combination of perchlorate and methimazole is efficacious in the treatment of AAT in patients with goiters.

MATERIALS AND METHODS

Patients

Studies were carried out in 23 patients with AAT (13 males, 10 females, aged 32-77 yr) residing in West Tuscany, Italy, a region of mild iodine deficiency. They had received long-term treatment with amiodarone (mean 17 months, range 4-60 months) at a dose of 1000-2800 mg/week for tachyarrhythmias or ischemic heart disease. Twenty patients were found to be hyperthyroid during amiodarone administration and amiodarone was discontinued when the diagnosis was established. AAT appeared 2-3 months after withdrawal of the drug in the other 3 patients. The diagnosis of hyperthyroidism was established in all patients by the finding of an elevated free T_3 index (FT3I) and clinical manifestations of thyrotoxicosis including weight loss, sweating, nervousness, tremor and increasing heart rate despite amiodarone administration. Nine patients with

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Key words: Amiodarone, iodine, methimazole, potassium perchlorate, hyperthyroidism

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hyperthyroidism were followed monthly for 6-10 months without antithyroid drug therapy (Group A). The remaining 14 patients were divided into two groups. Six patients were given 20 mg methimazole (MMI) twice daily (Group B). Since this proved to be ineffective in inducing euthyroidism, 8 subsequent patients were given the same dose of MMI and potassium perchlorate ($KClO_4$), 1.0 g/daily (Group C). Two patients in the latter group had been unsuccessfully treated with MMI (40 mg/day) and prednisone (60 mg/day) for one month. $KClO_4$ was withdrawn as soon as the patients reached the euthyroid state and in no case was the drug continued for more than 40 days. Patients receiving $KClO_4$ were monitored with complete blood counts and urinalysis.

Biochemical Studies

Total T_4 (TT_4) and total T_3 (TT_3) were assayed by RIA using commercial kits (T_4 RIA and T_3 RIA Kits ARIA II, Becton Dickinson Laboratory System, Milan, Italy). Free T_4 index (FT_4I) and free T_3 index (FT_3I) were calculated as the product of the T_3 resin uptake (Trilute Kit, Miles Italiana S.p.A. Ames Division, Milan, Italy) and the TT_4 and TT_3, respectively; serum thyrotropin (TSH) was assayed by a commercial kit (Byk-Mallincrodt S.p.A., Milan, Italy). Antithyroglobulin and antimicrosomal antibodies were determined by passive hemagglutination (Thyroid Test Kit and Microsome Test Kit, Fujizoki Pharmaceutical Co., Tokyo, Japan). The urinary excretion of iodine was measured by the modified method of Zak using an autoanalyzer Technicon apparatus (24).

Table 1 - *Baseline studies in patients with amiodarone iodine induced thyrotoxicosis (AAT).*

Patient no	Age/sex		Receiving amiodarone at time of dx	TT_4 (μg/dl)	TT_3 (ng/dl)	FT4I	FT3I	TSH (μU/ml)	AbTg	AbM	Thyroid Examination
Group A											
1	56	F	Yes	15.7	321	23.0	471	< 0.5	neg.	neg.	Normal
2	68	F	Yes	11.0	450	14.9	450	< 0.5	neg.	neg.	Normal
3	77	M	Yes	17.0	289	16.0	271	< 0.5	neg.	neg.	Normal
4	39	F	Yes	21.3	259	26.1	318	< 0.5	neg.	neg.	Normal
5	73	M	Yes	17.2	410	18.8	447	< 0.5	neg.	1:1.600	Normal
6	46	M	Yes	15.9	255	16.7	268	< 0.5	1:100	1:25.600	Diffuse goiter
7	50	F	Yes	12.7	234	12.0	223	< 0.5	neg.	neg.	Multinodular goiter
8	68	M	Yes	27.0	203	38.3	288	< 0.5	neg.	neg.	Multinodular goiter
9	44	F	Yes	12.2	345	13.7	379	< 0.5	neg.	neg.	Multinodular goiter
	Mean ± SE			16.7 ± 1.6	307.3 ± 27.4	19.9 ± 2.7	346.1 ± 30.9				
Group B											
10	44	M	Yes	17.4	284	23.1	378	< 0.5	neg.	neg.	Normal
11	36	M	Yes	30.5	277	39.6	363	< 0.5	neg.	neg.	Diffuse goiter
12	58	F	3*	27.0	450	27.0	450	< 0.5	neg.	neg.	Diffuse goiter
13	52	M	Yes	14.0	293	17.2	359	< 0.5	neg.	neg.	Multinodular goiter
14	70	M	Yes	11.0	294	18.0	482	< 0.5	neg.	neg.	Multinodular goiter
15	61	F	Yes	20.9	226	30.7	331	< 0.5	neg.	neg.	Multinodular goiter
	Mean ± SE			20.1 ± 3.1	304 ± 30.9	25.9 ± 3.4	393.8 ± 24				
Group C											
16	32	M	Yes	13.1	356	17.7	485	< 0.5	neg.	neg.	Normal
17	59	M	3*	25.4	306	36.2	429	< 0.5	neg.	neg.	Normal
18	47	M	Yes	17.6	466	25.0	662	< 0.5	1:100	1:6.400	Diffuse goiter
19	62	F	2*	16.5	249	20.7	312	< 0.5	neg.	1:6.400	Diffuse goiter
20	57	M	Yes	30.0	349	36.6	437	< 0.5	neg.	neg.	Multinodular goiter
21	52	M	Yes	21.4	390	23.0	418	< 0.5	neg.	neg.	Multinodular goiter
22	54	F	Yes	15.5	215	21.6	300	< 0.5	neg.	neg.	Multinodular goiter
23	56	F	Yes	14.7	266	18.7	338	< 0.5	neg.	neg.	Single hot nodule
	Mean ± SE			19.3 ± 2.1	324.6 ± 29.1	24.9 ± 2.6	422.6 ± 41.4				
Normal range				4.2-12.0	100-210	4-11	100-208	0.5-3.8			

*Number of months off amiodarone at time of diagnosis of AAT.

Diagnosis of Amiodarone Associated Thyrotoxicosis

Since TT_4 and FT_4I may be elevated and TT_3 and FT_3I are always normal or low in euthyroid patients chronically treated with amiodarone (9, 11, 18), the diagnosis of AAT was made on the basis of elevated serum TT_3 and FT_3I and undetectable serum thyrotropin (TSH) concentrations in the presence of clinical evidence of hyperthyroidism (18). The urinary iodine excretion was high in all subjects at the time of diagnosis, ranging from 700 to 4500 μg iodine/g creatinine. Urinary iodine excretion in normal, euthyroid subjects residing in West Tuscany, Italy, and not receiving iodine containing drugs averages 77.8 μg iodine/g creatinine (range 55-130).

Statistical Analysis

Comparisons between the mean serum TT_4, FT_4I, TT_3 and FT_3I concentrations were made by analysis of variance (ANOVA). Elsewhere, the Fisher exact probability test (25) was used.

RESULTS

Baseline Studies and Thyroid Examination (Table 1)

Multinodular goiter was present in nine, a diffuse goiter in five, uninodular goiter in one, and an absence of detectable goiter in eight of the 23 patients with AAT at the time of diagnosis. Four patients had positive antimicrosomal antibodies, suggesting underlying autoimmune thyroid disease. The patients were all clinically thyrotoxic and serum TT_3 was elevated in all except 1 patient while FT_3I was elevated in all 23 patients. Although serum TT_4 or FT_4I were elevated in all patients, these thyroid function tests may also be elevated in euthyroid patients receiving amiodarone (8-13). There was no significant difference in mean serum TT_4, FT_4I, TT_3 and FT_3I values in the 3 groups.

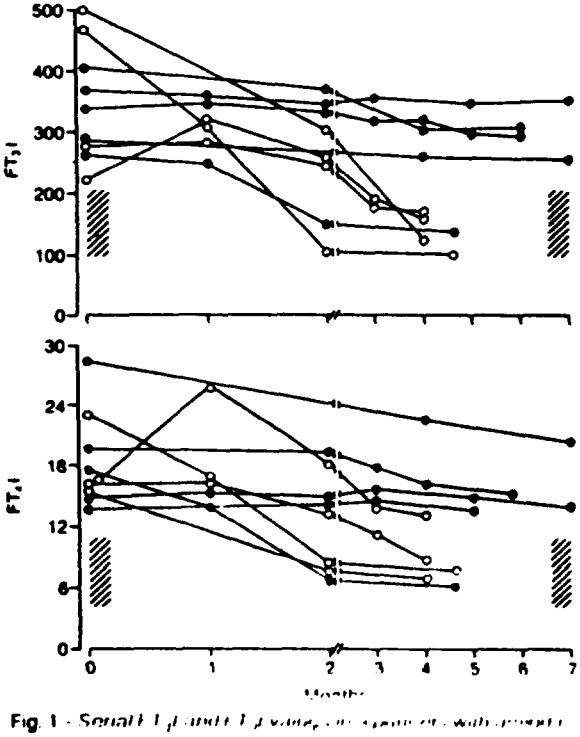

Fig. 1 - *Serial FT_4I and FT_3I values [illegible] with amiodarone associated thyrotoxicosis who [illegible] drug therapy. Closed and open circles [illegible] with or without underlying thyroid [illegible]. The shaded bars represent the normal ranges for FT_4I and FT_3I.*

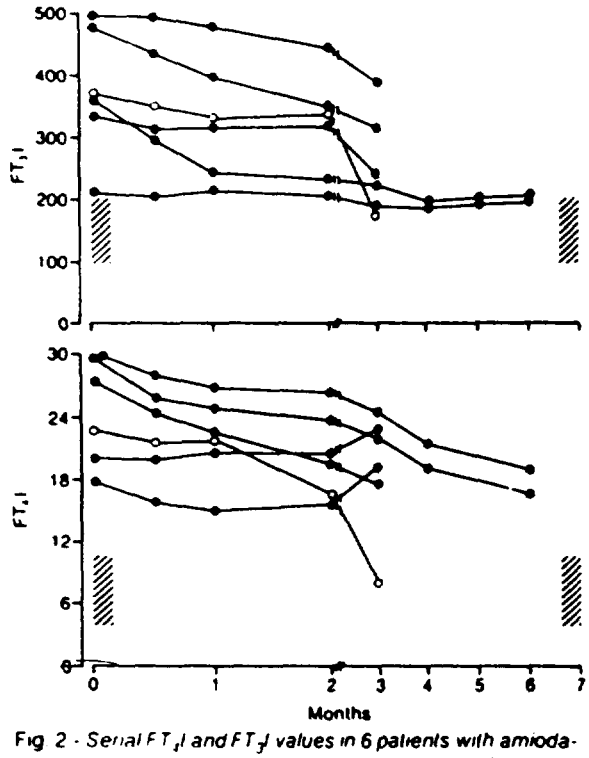

Fig. 2 - *Serial FT_4I and FT_3I values in 6 patients with amiodarone associated thyrotoxicosis treated with methimazole. Closed and open circles represent patients with or without underlying thyroid disease, respectively. The shaded bars represent the normal ranges for FT_3I and FT_4I.*

The Course of Thyroid Function in Untreated Patients (Group A, Fig. 1)

Following the diagnosis of AAT, amiodarone was discontinued in all 9 patients in this group. The 4 patients with goiter were still hyperthyroid 6-7 months later while the 5 patients without goiter (one of whom had positive antimicrosomal antibodies, patient no. 5) became clinically and biochemically euthyroid within 2-4 months (goiter vs nongoiter, remission < 4 months, $p < 0.01$).

The Effect of MMI Therapy (Group B, Fig. 2)

In spite of the administration of MMI (40 mg daily), the 5 patients with goiter remained clinically and biochemically hyperthyroid during the time they were observed

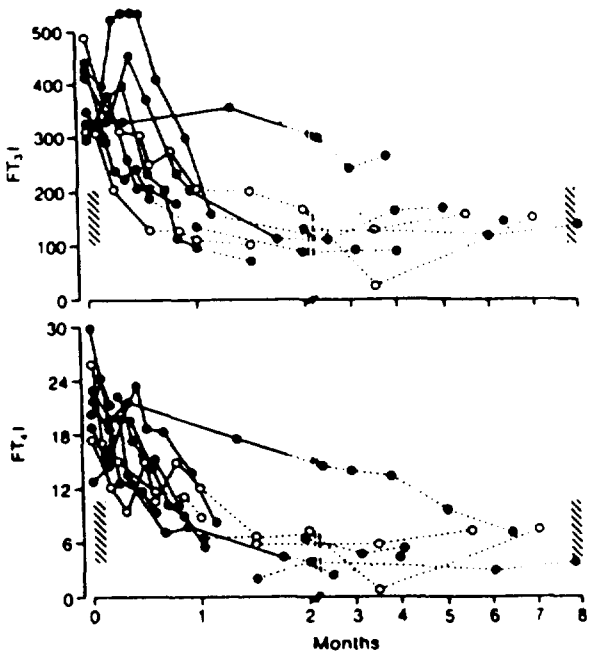

Fig. 3 - *Serial FT_4I and FT_3I values in 8 patients with amiodarone associated thyrotoxicosis treated with methimazole plus $KClO_4$. The duration of $KClO_4$ therapy is represented by the solid lines. The dotted lines represented values after $KClO_4$ was discontinued. The 2 patients without underlying thyroid disease (o) did not receive any additional antithyroid drug therapy, while patients with underlying thyroid disease (●) required 5-20 mg/methimazole daily to maintain the euthyroid state.*

on therapy (3 patients, 3 months and 2 patients, 6 months). The only patient in this group without goiter and with negative antibodies became euthyroid after 3 months of therapy (patient no. 10).

The Effect of Combined MMI and $KClO_4$ Therapy (Group C, Fig. 3).

In 7 patients, combined therapy with MMI (40 mg daily) and $KClO_4$ (1 g daily for 16-36 days) resulted in a rapid fall of serum FT_4I and FT_3I into the normal range and the restoration of clinical euthyroidism within 16-36 days after therapy was instituted. Included in this group were 2 patients (no. 17 and 22) who failed to respond to a previous one month course of therapy with prednisone and MMI. The excellent therapeutic response occurred in patients with or without goiter. In the remaining patient in this group (no. 21), thyrotoxicosis persisted for 40 days at which time $KClO_4$ was discontinued because of mild neutropenia but MMI was continued. This patient became euthyroid three and one-half months later. Thus, five of 6 patients with goiter treated with MMI and $KClO_4$ became euthyroid within 36 days of the institution of therapy while no patient with goiter treated with MMI alone became euthyroid before 3 months ($p < 0.01$).

After $KClO_4$ was discontinued, the two patients (no. 16 and 17) without goiter and with negative antibodies remained euthyroid for at least 2-4 months without MMI therapy. In contrast, the 6 patients with goiter required 5-20 mg MMI daily to maintain the euthyroid state during the period of observation. In three of these patients, MMI was discontinued along with $KClO_4$ and thyrotoxicosis recurred within 2 to 3 weeks requiring the reinstitution of MMI therapy. The remaining three patients were maintained continuously on MMI.

Sequential measurements of urinary iodine excretion immediately prior to and during antithyroid drug therapy were carried out in 6 patients receiving MMI and $KClO_4$ and in 2 patients receiving MMI alone. Urinary iodine excretion progressively decreased in all patients receiving combined drug therapy, reaching normal values in five of the 6 patients by 1 to 3 months of therapy and decreasing markedly in the reamaing patient (Fig. 4). Iodine excretion in five of these patients transiently increased between 2 and 15 days after combined therapy was instituted. In contrast, urinary iodine excretion remained elevated in both patients

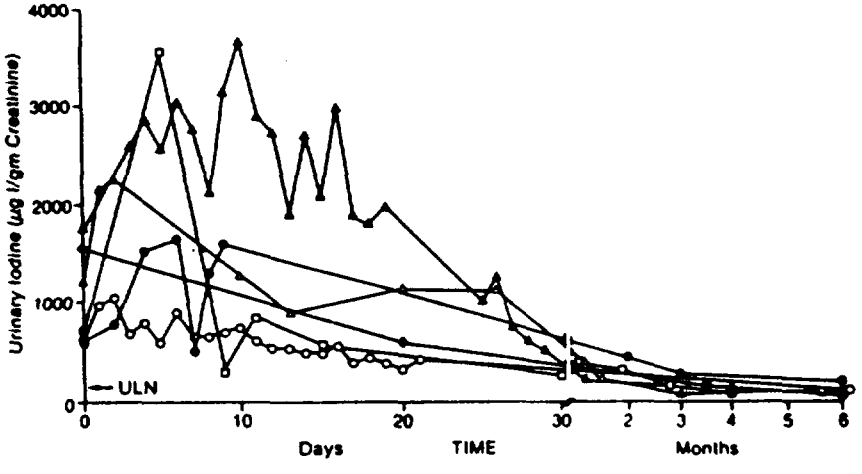

Fig. 4 - *Serial values of urinary iodine excretion expressed as µg iodine/g creatinine in 6 patients with amiodarone associated thyrotoxicosis treated with methimazole plus $KClO_4$. The 6 different symbols represent individual patients. ULN is upper limit of iodine excretion in normal subjects.*

treated with MMI alone. After 6 months of therapy, urinary iodine excretion was 325 and 1000 μg iodine/g creatinine in these two patients.

Except for mild transient leukopenia in one patient and mild transient epigastric distress in 3 patients, no adverse side effects were observed in the 8 patients treated with $KClO_4$.

DISCUSSION

In the present study, the course of AAT following withdrawal of amiodarone was evaluated in patients not receiving antithyroid drug therapy and in those treated with 2 different antithyroid drug regimens. The course of the patients with AAT who did not receive antithyroid drugs is similar to that reported by others (19, 20, 22), suggesting that AAT may spontaneously remit within a few months in some patients. However, it should be emphasized that this remission does not occur in all such patients. In addition, our study indicates that the coexistence of underlying thyroid disease adversely affects the natural history of AAT. Thus, four of the 5 untreated patients who became euthyroid 2-4 months after diagnosis had no apparent underlying thyroid disease, while all four patients with goiter remained thyrotoxic for at least 6-8 months.

The results obtained in the six patients with AAT treated with high doses of MMI are in agreement with previous observations, suggesting that this type of thyrotoxicosis is relatively resistant to thionamides (19-23). Five patients, all with underlying thyroid disease, treated with 40 mg MMI daily remained clinically and biochemically thyrotoxic for 3-6 months. The one patient in this group who became euthyroid after 3 months of MMI therapy had no evidence of coexistent thyroid disease, suggesting the possibility of a spontaneous remission rather than a specific effect of MMI therapy.

It has been reported that the administration of prednisone with (20) or without (23) carbimazole induced a rapid remission of AAT. In the present study, this combined therapy was ineffective in 2 patients. The reason for this discrepancy is unclear. Further studies with a larger number of patients are required in order to determine the effectiveness of thionamide and corticosteroid therapy in patients with AAT.

Potassium perchlorate depresses thyroid hormone synthesis by competitively inhibiting iodide uptake by the thyroid gland. This drug was used in the 1950's for the treatment of hyperthyroidism (26, 27). The use of this anion in combination with MMI had been advocated for thyrotoxicosis (28). However, prolonged administration of $KClO_4$ was subsequently abandoned since severe toxic reactions such as fatal aplastic anemia and nephrotic syndrome were occasionally observed (29, 30). These severe complications were almost always associated with large doses of $KClO_4$ (more than 1 g daily). However, daily doses of 1 g or less have recently been reported to be successful in the long-term treatment of Graves' disease without serious side effects (31). In the present study, we considered that the potential risk of severe AAT in cardiac patients justified a short-term trial of $KClO_4$ therapy (1 g daily for a maximum of 40 days) under very careful hematologic and urinary monitoring. Except for 1 patient with transient neutropenia, no side effects of $KClO_4$ were observed. All but one of the 8 patients who received combined MMI and $KClO_4$ therapy rapidly became euthyroid by clinical and biochemical parameters within 2-4 weeks of the institution of therapy. The decrease in serum thyroid hormone concentrations was similar in patients with or without evidence of underlying thyroid disease. Follow-up study after withdrawal of $KClO_4$ suggests that the patients without apparent coexistent thyroid disease remained euthyroid without further treatment, while those patients with goiter, with or without positive thyroid antibodies, required low doses of MMI to maintain the euthyroid state. The present findings suggest that the short-term administration of $KClO_4$ reduces the prolonged refractoriness to thionamides observed in many patients with AAT treated with MMI alone.

The mechanism of the synergistic action of $KClO_4$ with MMI in the therapy of AAT remains unclear. $KClO_4$ could exert its action by inducing a rapid depletion of excess intrathyroidal iodine whose organification is blocked by the concomitant administration of MMI. $KClO_4$ would also block further entrance of iodide into the thyroid since it is a potent inhibitor of the active transport of iodide from plasma into the thyroid. In keeping with this concept was the frequently observed rapid increase in urinary excretion followed by a progressive decrease in patients treated with MMI and $KClO_4$. This is in contrast with the persistently high urinary iodine excretion observed in the two patients treated with MMI alone. Although the mechanism by which amiodarone induces thyrotoxicosis is not completely clear, it seems likely that the high iodine content of the drug plays a crucial role (15). This concept is further supported by recent data using fluorescent thyroid scans in patients receiving long-term amiodarone therapy (21). Leger et al. reported that the thyroid iodine content in AAT was elevated only during the thyrotoxic phase, returning to normal after remission of the hyperthyroidism. Interestingly, thyroid iodine content in euthyroid amiodarone treated patients was normal. On the basis of this finding, these Authors speculated that thyrotoxicosis in amiodarone treated patients is the consequence of a failure of the inhibitory effect of excess iodide on the organification of iodide resulting in a subsequent increase in thyroid hormone synthesis (21). Another explanation could be that the thyroid iodide trap, which normally decreases in the presence of excess plasma iodide, thereby protecting the thyroid against excessive intrathyroidal iodine, fails to decrease in patients who develop AAT.

The results of the present study suggest that AAT may spontaneously remit and that this occurs more fre-

quently in patients without underlying thyroid disease. Thus, careful observation of these patients, perhaps employing beta blocking drugs, may be considered in patients with thyrotoxicosis and no evidence of goiter. Thionamides alone appear to be relatively ineffective in the treatment of AAT, especially in the presence of goiter. The short-term administration of $KClO_4$ in association with MMI appears to be effective in rapidly controlling the thyrotoxicosis. Due to the potential toxicity of $KClO_4$, its use should be reserved for those patients with clinically severe AAT and careful monitoring of potential side effects should be carried out.

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LETTER TO THE EDITOR

More on $KClO_4$ and amiodarone associated thyrotoxicosis

Sir,

In areas of mild iodine deficiency thyrotoxicosis is frequently observed in patients receiving chronic amiodarone therapy (1). The pathophysiology of amiodarone associated iodine induced thyrotoxicosis (AAT) is not fully understood and its therapy has been long disappointing. Recently Martino et al. (2) reported the successful treatment of 7 on 8 cases of AAT with the simultaneous administration of $KClO_4$ and methimazole (MMI). The paper of Martino et al. represents undoubtedly an important progress in the treatment of AAT, but some problems remain open. A major one is: when to stop $KClO_4$ treatment? Both MMI and $KClO_4$ have a potential bone marrow toxicity; they should therefore be used under careful hematologic monitoring only in severe cases of AAT. The concern about toxicity suggests as short courses of therapy as possible, but the mere normalization of T_3 and T_4 does not seem to be a sufficient criterium to insure safe $KClO_4$ withdrawal, exposing to the risk of AAT relapse (3).

We report here the case of a 66-yr-old man with an ischemic cardiomyopathy and episodes of ventricular tachyarrhythmia which had been treated since 1984 with amiodarone 200 mg/d. Before the treatment T_3 was 93 ng/dl (normal 80-220) and T_4 was 9.2 μg/dl (normal 5-12). After 40 months of therapy he developed atrial fibrillation and severe weight loss. He complained also of discomfort in the lower anterior region of the neck, but no goitre was revealed by echography. T_3 was 115 ng/dl and T_4 13.8 μg/dl. An AAT was diagnosed and amiodarone was withdrawn. MMI 15 mg/d was ordered, but one month later T_3 and T_4 had reached respectively 270 ng/dl and 26 μg/dl. MMI was then increased to 30 mg/d and $KClO_4$ 800 mg/d was added. In two weeks T_3 fell to 180 ng/dl and T_4 to 18 μg/dl, and $KClO_4$ was withdrawn. A week later T_3 had increased to 185 ng/dl. $KClO_4$ was so readministered for ten more days, until T_3 reached 114 ng/dl and T_4 11.5 μg/dl. $KClO_4$ was then definitively withdrawn. MMI was gradually reduced and then stopped, without recurrence of thyrotoxicosis. No hematologic disturbances were observed, and the patient's sinus rhythm was restored by means of an electric cardioversion.

AAT is most likely due to excessive intrathyroidal iodine content. As Martino et al. (2) pointed out, the simultaneous administration of $KClO_4$ and MMI rapidly removes the iodine surcharge from the thyroid. MMI blocks in fact iodine organification while $KClO_4$ concomitantly exerts a rapid depletion of the intrathyroidal iodine overload and prevents further entrance of iodine into the thyroid. A premature withdrawal of $KClO_4$ may lead to the recurrence of AAT probably because the iodine excess has not yet completely been eliminated (3). In that case a second brief course of therapy with $KClO_4$, inducing further elimination of iodine, may obtain the definitive control of AAT, as shown by the case of our patient. So the almost complete elimination of iodine overload seems to be needed to avoid AAT relapse. In this sense, in patients treated with $KClO_4$ and MMI, the progressive decrease that follows the initial increase in urinary iodine excretion could indicate that not only the intrathyroidal, but also the extrathyroidal iodine excess has been eliminated. Could this decrease in urinary iodine excretion be the missing clinical clue to a timely and safe $KClO_4$ withdrawal?

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This letter was forwarded to Dr. E. Martino whose reply follows:

Sir,

We read carefully the interesting letter by Dr. Dal Fabbro and co-workers regarding the use of simultaneous

administration of $KClO_4$ and methimazole (MMI) for the treatment of amiodarone-iodine-induced thyrotoxicosis (AITT). We are pleased to realize that this therapeutic regimen was effective in controlling the thyrotoxicosis in the case reported. As pointed out by the Authors, however, the important clinical question of when $KClO_4$ should be withdrawn is still open. In our series(1) $KClO_4$ was interrupted as soon as the patients reached clinical and biochemical evidence of euthyroidism (corresponding to a period of combined treatment of 19-42 days) and no subsequent recurrence of thyrotoxicosis was observed. In the case reported by Dal Fabbro et al., the first course of $KClO_4$ + MMI therapy was rather short (2 weeks) and $KClO_4$ was withdrawn when serum thyroid hormone concentrations were still elevated, indicating that euthyroidism was not yet achieved. Their suggestion that normalization of iodine urinary excretion could be a good indicator for a "timely and safe $KClO_4$ withdrawal" is certainly pertinent and interesting. In all our patients, however, the urinary iodine excretion (although reduced with respect to the pre-treatment values) was still very high when $KClO_4$ was stopped, and waiting for normalization of urinary iodine excretion would have implied more prolonged $KClO_4$ administration, with increased probability of drug toxicity. A difficulty encountered in treatment of AIIT with combined $KClO_4$ and MMI was the variability in the time needed for a good therapeutic response observed in the individual patients. In our experience patients with underlying thyroid disorders required more prolonged treatment when compared to those with AIIT without other apparent thyroid abnormalities. Although the precise mechanism responsible for this phenomenon is still unknown, it has been recently shown that the presence of underlying thyroid disorders markedly affects the absolute intrathyroidal iodine content in amiodarone-treated patients in spite of similar degree of extrathyroidal iodine overload (2).

In conclusion, although sequential determination of urinary iodine excretion is an extremely useful tool in the follow-up of AIIT patients to quantitate the degree of iodine overload, it would appear that selection of the most convenient time of $KClO_4$ withdrawal is based on the achievement of clinical and biochemical evidence of euthyroidism.

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LETTER TO THE EDITOR

Delayed control of iodine-induced thyrotoxicosis with a thionamide after $KClO_4$ withdrawal

Sir,

In a recent letter, De Weweire et al. (1) reported worsening of biochemical hyperthyroidism after withdrawal of $KClO_4$ (administered for 8 days) despite the maintenance of high doses of methimazole (MMI), in a patient with amiodarone associated thyrotoxicosis (AAT). This observation contrasted with the report of Martino et al. (2) whose patients with AAT were treated with MMI and $KClO_4$ during 40 days and remained euthyroid with MMI alone after $KClO_4$ withdrawal.

Our patient, a 60-year-old woman, had been treated for months with an iodine-containing linctus for bronchial asthma. She lost 30 kg in 6 months before to consult. Clinical hyperthyroidism (tachycardia, finger tremor, sweating hands and multinodular goiter) was confirmed by biochemical data (Fig. 1). The patient was treated with propylthiouracil (PTU) 400 mg/day and $KClO_4$ 900 mg/day for 40 days with a rapid and sharp decrease of serum free thyroxine (FT_4). Serum triiodothyronine (T_3) decreased slightly. After $KClO_4$ withdrawal both serum FT_4 and T_3 increased over a 2-week period. Afterwards, their serum levels decreased again but more slowly than during the period of treatment with PTU and $KClO_4$, and returned to normal values within 3 months.

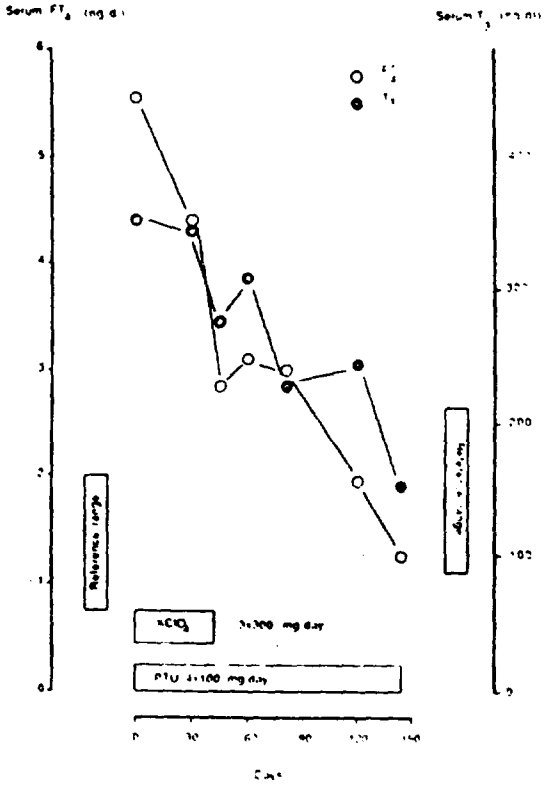

Fig. 1 - *Modifications of serum FT_4 and T_3 in a patient with iodine-induced thyrotoxicosis treated with $KClO_4$ and PTU*

Our observation showed that the association of PTU and $KClO_4$ is successfully used in the treatment of hyperthyroidism induced by iodinated drugs. PTU was preferred to MMI because of its peripheral inhibition of T_4 to T_3 conversion. However, euthyroidism was not obtained after 40 days of treatment and, after $KClO_4$ withdrawal, serum T_3 and FT_4 increased for a short period. In addition, with PTU alone, the slope of the decreasing curve of serum T_3 and FT_4 was obviously less sharp than that observed with PTU plus $KClO_4$ (Fig. 1). These data confirm the usefulness of thionamide and $KClO_4$ association in the treatment of iodine-induced thyrotoxicosis and the synergistic action of both drugs provided they were administered for up to 40 days, as suggested (2).

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J Endocrinol Invest. *9*: 201, 1986.

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NJM 00650

Brief Report

Treatment of amiodarone-induced hypothyroidism with potassium perchlorate

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The antiarrhythmic drug, amiodarone, induces thyroid dysfunction, which is potentially dangerous in cardiac patients. After discontinuation of the drug it takes several months before euthyroidism is restored. The potent antithyroid drug, potassium perchlorate ($KClO_4$), is used successfully to treat amiodarone-induced thyrotoxicosis, but it is less well known as potential treatment in amiodarone-induced hypothyroidism. In this case report we describe the successful use of two courses of $KClO_4$ treatment in a cardiac patient with severe amiodarone-induced hypothyroidism. The mechanisms responsible for the amiodarone-induced hypothyroidism and rationale for the use of $KClO_4$ in this condition are discussed.

Key words: Amiodarone; Thyroid dysfunction; Hypothyroidism; Perchlorate

Introduction

Amiodarone (AM), introduced for the treatment of ischaemic heart disease, is often used to treat refractory cardiac arrhythmias [1]. Although many adverse reactions have been reported [2], it is also an interesting drug for internists because of its complex effects on thyroid function [3]. One tablet of 200 mg AM contains 74.4 mg iodine of which about 10% is liberated in vivo [4]. These pharmacological doses of iodine (the optimal iodine intake, as recommended by the WHO, is 0.1–0.3 mg/day), can affect thyroid hormone production [5]. AM-induced thyroid disorders are difficult to diagnose because AM also has a direct inhibitory effect on the 5' deiodination of the iodothyronines, resulting in a decreased plasma concentration of triiodothyronine (T3) and increased plasma concentrations of thyroxine (T4) and reverse triiodothyronine (rT3) [6]. Abnormal thyroid function is not a rare event during AM treatment. A recent prospective study showed development of thyrotoxicosis in 12.1%, and of hypothyroidism in 6.9% of AM-treated patients [7]. The latter occurs early in the course of AM medication, especially in females with thyroid auto-antibodies prior to treatment [7,8]. AM has a long half-life, probably due to storage in many tissues. After discontinuation of the drug it can take several months before restoration of the euthyroid state, a potentially dangerous period in cardiac patients [9]. Martino et al. reported that patients with AM-induced thyrotoxicosis or hypothyroidism could be treated successfully with potassium perchlorate ($KClO_4$) [9,10]. Treatment

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of AM-induced thyrotoxicosis with $KClO_4$, a potent antithyroid drug, has been reported by others, but the use of $KClO_4$ in AM-induced hypothyroidism is less well known [10]. In this case report we will outline the presumed pathogenesis of AM-induced hypothyroidism and the mechanism of action of $KClO_4$.

Case Report

A 63-yr-old man was admitted to the hospital because of an exacerbation of his chronic bronchitis. In the previous four months he had complaints of increasing fatigue, mental lethargy, cold intolerance and constipation. His weight had increased by 5 kg. Nine months earlier a diagnosis of ischaemic heart failure had been made elsewhere; the treatment prescribed was 100 mg AM once daily, the cumulative dose of AM upon admission being 27.3 g. Until admission he experienced no further signs of ischaemic heart disease. There was no personal or family history of thyroid disease.

On physical examination, he appeared lethargic and his speech was hoarse. Blood pressure and pulse rate were normal and his thyroid gland was not enlarged. There were symptoms of chronic bronchitis; no friction rub or murmurs were audible.

An X-ray of the chest showed an enlarged heart, but no signs of heart failure. On ultrasonography there was global left ventricular hypokinesia and slight pericardial effusion. Electrocardiography showed sinus rhythm with first degree atrioventricular block and a complete left bundle branch block.

Serum thyroid stimulating hormone (TSH, determined by immunoradiometric assay) was elevated (55.4 mU/l; N: 0.2–3.5 mU/l), with low free T4 (FT4: 2.2 pmol/l; N: 9.0–26.0 pmol/l) and total T3 levels (0.4 nmol/l; N: 1.0–3.0 nmol/l). No anti-microsomal or anti-thyroglobulin autoantibodies could be shown. Thyroid radioactive iodine (^{123}I) uptake studies revealed a high 1-h thyroidal uptake of 29% of the administered dose. Three hours after the administration of 1 g potassium perchlorate, the uptake had fallen to 3.3%.

A diagnosis of AM-induced hypothyroidism was made and AM was discontinued. In view of the short duration, it was felt safe to rapidly treat the severe hypothyroidism. The patient was therefore given 500 mg of $KClO_4$ twice daily for 3 wk, which resulted in rapid normalization of thy-

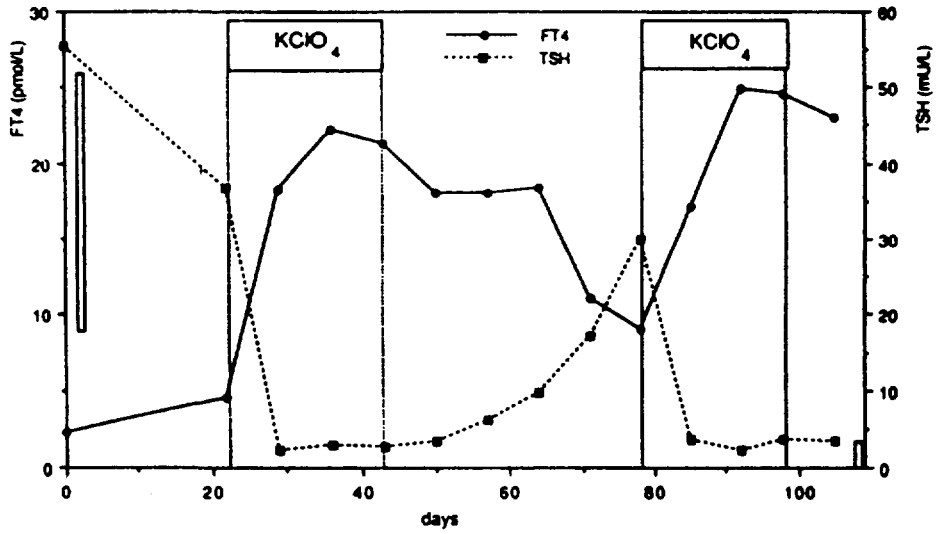

Fig. 1. The course of free T4 (FT4) and TSH levels in a 63-yr-old man with amiodarone-induced hypothyroidism, treated with potassium perchlorate ($KClO_4$). Reference ranges are indicated by bars (TSH-IRMA 0.2–3.5 mU/l, FT4 9.0–26.0 pmol/l).

roid function and disappearance of signs and symptoms. Four weeks after discontinuation of $KClO_4$ a biochemical relapse occurred. After another 3 wk of treatment with 250 mg of $KClO_4$ twice daily, rapid and lasting restoration of thyroid function followed (Fig. 1). Careful monitoring revealed no signs of toxicity nor coronary ischaemia. One year later the patient was still euthyroid.

Discussion

Using AM, the patient was exposed to large quantities of iodine. Exposure to iodine excess does not usually result in sustained thyroid dysfunction because of several adaptive processes. First, transport of large quantities of iodide into the thyroid is followed by inhibition of hormone synthesis: the Wolff-Chaikoff effect [11]. The mechanism responsible for this phenomenon has not yet been fully clarified, but depends upon high intrathyroidal iodide concentration. It has been suggested that the acute inhibition is due to complexing of oxidized iodide in the presence of high concentrations of inorganic iodide to yield I_3^-, which cannot be used in iodination reactions. Secondly, it has been suggested that there is a specific iodinated inhibitor of iodide transport, a hypothetical compound X-I [12], the concentration and action of which are supposed to vary with the total organic iodine content of the thyroid. A high thyroidal iodide concentration will lead to formation of this hypothetical compound X-I, which inhibits iodide transport (Fig. 2), resulting in a fall in thyroid iodide content and mitigation of the inhibition of iodothyronine synthesis. This latter phenomenon might be involved in the escape from the Wolff-Chaikoff effect [11]. Thirdly, the presence of large quantities of iodide retards the rate of hormone secretion through an inhibition of proteolytic release of iodothyronines from thyroglobulin. As in the case of the Wolff-Chaikoff effect, escape from this effect also occurs when the intrathyroidal iodide content falls as a result of autoregulatory inhibition of iodide transport [13].

AM-induced hypothyroidism is assumed to result from a failure of the thyroid to escape from

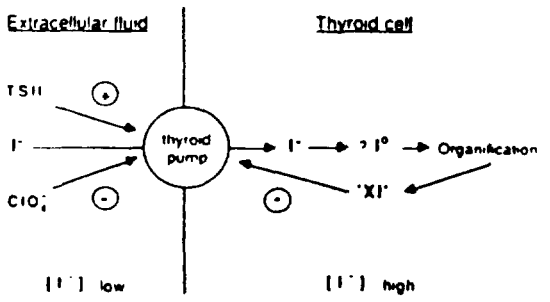

Fig. 2. Schematic drawing, representing the active transport of iodide into the thyroid follicular cell which is accomplished by the "thyroid-pump". This pump is stimulated by TSH and proposed to be inhibited by an unkown iodinated compound X-I, the concentration of which varies with the total organic iodine content of the thyroid. $KClO_4$ blocks this pump, permitting efflux of iodide from the thyroid cells.

the inhibitory effect of iodide on organification as a consequence of uninhibited iodide trapping. This hypothesis is supported by the preserved radioiodide uptake, as demonstrated in our patient, despite dilution of the radioisotope by the increased iodide pool [14,15]. It is proposed that this ongoing iodide transport is caused by a defect in organification in patients with AM-induced hypothyroidism, resulting in insufficient formation of X-I. Perchlorate competitively inhibits iodide transport and thus rapidly depletes the thyroid gland of inorganic iodide. The positive perchlorate discharge test in our patient thus indicates that the accumulated ^{123}I was not organified. These findings are in agreement with the low organic iodine content in patients with AM-induced hypothyroidism, as compared to the markedly elevated levels of organified iodine in patients with AM-induced thyrotoxicosis [16,17]. Another possible mechanism, not mentioned previously, involves the TSH-receptor. TSH stimulates thyroidal iodide transport and might thus increase the thyroid iodide content. In vitro studies have shown that AM increases the number of high affinity TSH-binding sites and this is not seen during incubation with iopanoic acid [18]. This effect was already present at concentrations of AM which were unable to inhibit iodide uptake but nevertheless could significantly reduce iodide organification. By increasing the number of TSH-receptors, AM might, therefore, specifi-

cally stimulate iodide transport, thus perpetuating the Wolff-Chaikoff effect.

Whatever the exact mechanism, depleting intrathyroidal iodide stores seems rational in the treatment of AM-induced thyroid dysfunction. Perchlorate was formerly used to treat hyperthyroidism, but abandoned because of the occasional observation of fatal aplastic anaemia and development of a nephrotic syndrome. These severe complications were almost always associated with large doses of $KClO_4$ (more than 1 g daily) [19]. It was recently reintroduced by Martino et al., who successfully used the drug concomitantly with methimazole for the treatment of AM-induced thyrotoxicosis [9]. Paradoxically, it also appeared to be effective in 6 cases of AM-induced hypothyroidism reported by the same author [10]. In another report, however, only a transient effect was seen [20]. In our patient we diagnosed AM-induced hypothyroidism with a high uptake of ^{123}I and a positive $KClO_4$ discharge test. We demonstrated that two short courses of low dose $KClO_4$ treatment were effective and safe in rapidly restoring euthyroidism, although the potential toxic side effects require a limited application in time.

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Treatment of amiodarone induced hyperthyroidism with potassium perchlorate and methimazole during amiodarone treatment

Louis J M Reichert, Hans A M de Rooy

Abstract

To exploit the antiarrhythmic effect of amiodarone when patients develop the side effect of thyrotoxicosis three patients with hyperthyroidism induced by amiodarone were given simultaneously 1 g potassium perchlorate a day for 40 days and a starting dose of 40 mg methimazole a day while they continued to take amiodarone. As hyperthyroidism might have recurred after potassium perchlorate treatment was stopped the dose of methimazole was not reduced until biochemical hypothyroidism (raised thyroid stimulating hormone concentrations) was achieved. The patients became euthyroid (free triiodothyronine concentration returned to normal values) in two to five weeks and hypothyroid in 10 to 14 weeks. One patient became euthyroid while taking 5 mg methimazole a day and 600 mg amiodarone weekly; the two others required substitution treatment with thyroxine sodium while taking 5 mg methimazole or 50 mg propylthiouracil (because of an allergic reaction to methimazole) and 2100 or 1400 mg amiodarone weekly.

Hyperthyroidism induced by amiodarone may be treated with potassium perchlorate and methimazole given simultaneously while treatment with amiodarone is continued.

Introduction

Amiodarone is widely used as an antiarrhythmic drug to control dangerous cardiac irregularities. It has, however, a well recognised propensity to induce thyroid disturbances.[1] If hyperthyroidism is induced the patient's condition often becomes precarious, with deterioration in the effectiveness of amiodarone, and becomes worse when treatment with amiodarone is stopped. Nevertheless, stopping amiodarone treatment is almost universally accepted before trying to control the hyperthyroidism with antithyroid drugs. As amiodarone contains 37·5% iodine (75 mg iodine per 200 mg tablet) and has an elimination half life of two to three months, however, the thyroid will be overloaded with iodine when the hyperthyroidism occurs and antithyroid drugs might therefore be expected not to have any effect. The commonly observed low uptake of radioactive iodine by the thyroid in amiodarone induced hyperthyroidism prevents the use of iodine-131, and thyroidectomy is often too dangerous to be considered in patients with heart disease and uncontrolled hyperthyroidism. So it seems logical to remove the excess iodine from the thyroid and then start treatment with the antithyroid drug.

Martino *et al* gave their patients potassium perchlorate and methimazole simultaneously and they became euthyroid in two to five weeks, presumably by the above mechanism.[1] Martino *et al*, however, stopped treatment with amiodarone and therefore lost its beneficial antiarrhythmic effect. To be able to continue amiodarone treatment, however, would probably be potentially life saving in a small number of patients. We report on three patients with hyperthyroidism induced by amiodarone who were treated with potassium perchlorate and methimazole while they continued to take amiodarone.

Patients and methods

The patients were aged 59 (case 1), 71 (case 2), and 67 (case 3) and developed hyperthyroidism during treatment with amiodarone. They all had serious ischaemic heart disease; two patients (cases 1 and 2) had recurrences of dangerous cardiac irregularities, which had previously been responsive only to amiodarone, and developed heart failure. Amiodarone induced hyperthyroidism was thought to be the cause of the deterioration in all three patients, and because of their earlier responsiveness to amiodarone we decided not to stop treatment with amiodarone. Hyperthyroidism was diagnosed 38, 37, and 26 months after amiodarone treatment was started at respective doses of 2100, 1400, and 600 mg weekly. Amiodarone induced hyperthyroidism was diagnosed from the clinical pattern, a decreased thyroid stimulating hormone concentration, a raised free triiodothyronine concentration, and the absence of an image of the thyroid on scanning with technetium-99m. Thyroid antibodies were not detected in any of the patients.

Concentrations of free thyroxine and triiodothyronine were measured by radioimmunoassay (Amersham International, Buckinghamshire) and thyroid stimulating hormone concentrations by immunoradiometric assay (Boots, Celltech Diagnostics, Slough, Berkshire). Thyroid antibodies were determined by indirect immunofluorescence at the Central Laboratory of the Blood Transfusion Service in Amsterdam. Laboratory follow up included determination of thyroid stimulating hormone, free triiodothyronine, and free thyroxine concentrations, total blood counts, kidney function, and liver enzyme activities.

In addition to the treatment with amiodarone all three patients received 250 mg potassium perchlorate and 10 mg methimazole, both four times daily. In one patient (case 2) methimazole was stopped after one week and replaced a week later by 100 mg propylthiouracil four times daily because of an allergic reaction. The protocol was approved by the medical ethics committee of this hospital; the three patients gave their informed consent.

Results

On entry into the study all three patients had a noticeably decreased thyroid stimulating hormone concentration and a highly raised free triiodothyronine

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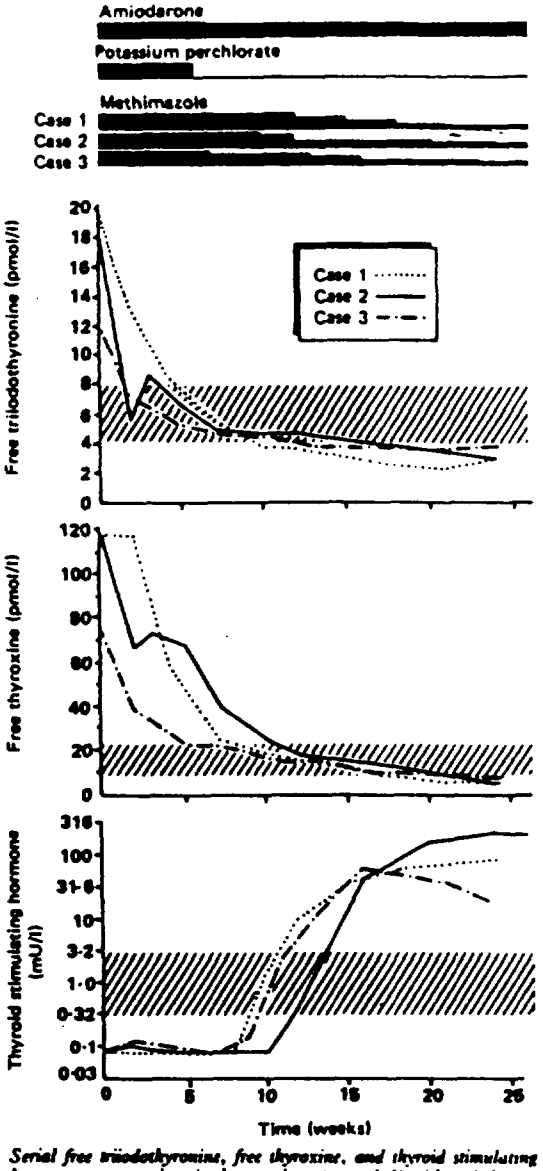
concentration. Although amiodarone treatment was continued, treatment with potassium perchlorate and methimazole resulted in the free triiodothyronine concentration returning to normal values after two weeks in one patient and after five weeks in the two others (figure). Free thyroxine, whose conversion to free triiodothyronine is inhibited by amiodarone,[4] returned to normal values after five to 10 weeks and thyroid stimulating hormone after eight to 12 weeks.



Serial free triiodothyronine, free thyroxine, and thyroid stimulating hormone concentrations in three patients (cases 1-3) with amiodarone induced hyperthyroidism during treatment with amiodarone and 1 g potassium perchlorate a day for 40 days and methimazole at starting dose of 40 mg a day. Dose of methimazole was gradually reduced but never completely stopped. Shaded areas represent normal ranges

Potassium perchlorate was discontinued after 40 days. After seven to 12 weeks the dose of methimazole was gradually reduced but never completely stopped. During the study no abnormalities were found in the complete blood counts, in the activities of liver enzymes, or in the results of kidney function tests.

One patient (case 3) became euthyroid when given 5 mg methimazole a day and 600 mg amiodarone weekly. The two other patients required substitution treatment with 100 µg thyroxine sodium a day while taking 5 mg methimazole (case 1) or 50 mg propylthiouracil (case 2) a day and 2100 or 1400 mg amiodarone weekly. The cardiac irregularities disappeared and the heart failure subsided. All three patients became well and resumed their activities.

Discussion

Various regimens of treatment have been described for hyperthyroidism induced by amiodarone. Discontinuation of amiodarone treatment lead to spontaneous euthyroidism only after two to eight months.[1] Martino *et al* reported good results with a combination of potassium perchlorate and methimazole after amiodarone treatment was discontinued.[2] Potassium perchlorate may cause the excretion of excess iodine and prevent the uptake of further iodine while methimazole inhibits its incorporation into triiodothyronine and thyroxine.

In 1952 Wyngaarden *et al* reported that potassium perchlorate interfered with the uptake of inorganic iodine by the thyroid gland in rats.[4] Because of side effects such as aplastic anaemia and the nephrotic syndrome, which occur predominantly at high doses or with prolonged administration, potassium perchlorate was no longer used for the treatment of hyperthyroidism.[5] During our study and that of Martino *et al* the patients received no more than 1 g potassium perchlorate a day for a maximum of 40 days and laboratory results showed that neither haematological nor renal complications occurred.

Our three patients became euthyroid (free triiodothyronine concentrations returned to normal values) within two to five weeks despite the continuation of amiodarone treatment. The same results were found when amiodarone treatment was discontinued before the hyperthyroidism was treated.[3]

Because of the fear that hyperthyroidism might recur during amiodarone treatment after potassium perchlorate treatment had been discontinued the dose of methimazole was not reduced until biochemical hypothyroidism (raised thyroid stimulating hormone concentrations) was achieved. This may be why our patients rapidly became hypothyroid. As a result we treated two other patients with the same regimen but reduced their dose of methimazole earlier; they became euthyroid and not biochemically hypothyroid within 12 and 18 weeks. Moreover, because of the fear that hyperthyroidism might recur we never completely stopped the antithyroid treatment, not even when the patients' hypothyroidism was sustained (cases 1 and 2). We preferred substitution treatment with thyroxine sodium because we were aware that the excess iodine was prevented from causing a recurrence of the hyperthyroidism only by the persistent (complete or partial) block of peroxidase activity by antithyroid treatment.

We conclude that hyperthyroidism induced by amiodarone may be treated with potassium perchlorate and methimazole during amiodarone treatment.

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Abrogation of c-MYC protein degradation in human lymphocyte lysates by prior precipitation with perchloric acid

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Conventional lysis buffers, though containing cocktails of protease inhibitors, did not prevent the degradation of c-MYC recombinant protein added immediately prior to lysis to cell pellets from human mixed lymphocyte cultures. Treatment of the cells with 4.2% perchloric acid, however, prevented protein degradation and facilitated the detection of c-MYC protein by Western blotting even in unstimulated lymphocytes, where previously it had been reported to be undetectable or barely detectable using this technique. PHA stimulation of lymphocytes induced an approximately six fold increase in measured c-MYC protein within 5 h if cell extracts were prepared using perchloric acid precipitation. However, using conventional lysis buffer the proto-oncogene protein was undetectable until 48–72 h after mitogen addition. Pretreatment with perchloric acid may be useful for Western blotting analysis of protein in other systems where it may be desirable to dispense with the use of toxic protease inhibitors or where these may be incompletely effective.

Key words: c-MYC protein; Lymphocyte; Western blotting

Introduction

Many authors have reported early transient increases in c-MYC mRNA, 3–5 h after stimulation of peripheral blood lymphocytes with mitogens in vitro (for example: Kelly et al., 1983; Reed et al., 1985; Lindsten et al., 1988.) However, there have been surprisingly few measurements of the actual c-MYC protein concentrations in such cells and no detailed time course studies have been performed. The reports of which we are aware are described below. Persson et al. (1984) observed a 20-fold excess of c-MYC protein compared with untreated controls 20 h after PHA stimulation of peripheral blood lymphocytes, in vitro, in which protein synthesis was measured by labelling for 18 h with [^{35}S]methionine prior to immunoprecipitation. Similarly, Moelling et al. (1984) found two proteins, immunoprecipitated by an anti c-MYC antibody, which were present in ConA stimulated lymphocytes at the single time point of 4 h after the addition of mitogen, but which were not detected in the untreated controls. Harel-Bellan et al. (1988) demonstrated that a c-MYC antisense

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Abbreviations: PHA, phytohaemagglutinin; PBS, phosphate-buffered saline; PMSF, phenylmethylsulphonyl fluoride; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulphate.

oligonucleotide could block the de novo synthesis of c-MYC protein in activated T cells, as measured by [^{35}S]methionine labelling for 3 h followed by two-dimensional electrophoresis and autoradiography. Heikkila et al. (1987) also showed that PHA stimulation of peripheral blood lymphocytes for 6 h caused an increase in c-MYC protein from undetectable to detectable levels as measured by Western blotting, immunohistochemistry and immunoprecipitation: accumulation of the protein was blocked by an antisense oligonucleotide. On the basis of the latter results we have investigated this experimental model in more detail, as a potential test system for the c-MYC antisense oligonucleotide analogues synthesised within our department. We have concentrated solely on measuring c-MYC protein since this is the actual functioning product of the gene, and according to Ferrari et al. (1990) the abundance of the mRNA and protein are not always linked in the case of c-MYC. As far as we are aware this is the first study to examine c-MYC protein levels in PHA-stimulated lymphocytes at time points beyond 30 h. The later times were of interest since our previous experiments had shown that the maximum proliferative response was at 72 h.

Initially c-MYC protein was only detected in cell lysates derived from lymphocytes which had been incubated with PHA for 48 h or more. At earlier times the cell lysis buffers used, though containing a cocktail of protease inhibitors, failed to prevent non-specific protein degradation. Boiling the extracts immediately after lysis improved c-MYC protein detection, but there was still a considerable loss of recombinant c-MYC protein internal standard added immediately prior to cell lysis. Pretreatment of cells with dilute perchloric acid, a technique used routinely for precipitating macromolecules and extracting nucleotides from cells (e.g., Tidd and Paterson, 1974) completely abrogated protein breakdown as measured by internal standard recovery and for the first time significant levels of c-MYC protein were observed in unstimulated lymphocytes, which had previously been reported to contain amounts undetectable by Western blotting analysis (Heikkila et al., 1987). Using perchloric acid pretreatment the pattern of c-MYC protein expression in PHA stimulated lymphocytes matched the early rise reported for c-MYC mRNA (Kelly et al., 1983).

Materials and methods

Human peripheral lymphocytes were separated from leukocyte enriched blood on Ficoll-Hypaque gradients and were incubated in RPMI medium plus 15% FCS for the duration of the experiments. Half of the lymphocytes were treated with PHA at optimal concentrations (2% PHA M Gibco) and half were untreated. Samples were taken for SDS-PAGE followed by general protein staining with Coomassie blue and Western blotting analysis 0, 5, 24, 48, and 72 h after PHA addition. Flow cytometric analysis of cell DNA content, as measured by propidium iodide fluorescence (Taylor, 1980), was used to check the degree of PHA stimulation, which was expressed as the percentage of cells present in the S, G2 and M phases of the cell cycle. Cells for electrophoresis and Western blotting analysis were spun down at 800g in a refrigerated centrifuge, washed in ice cold PBS and treated according to the following protocols.

(1) Lysis with cold lysis buffer (10% SDS, 10% glycerol, with the protease inhibitors, 0.1 mM leupeptin, 0.1 mM PMSF in 0.04 M Tris pH 6.8, 4°C) followed by shearing of released DNA by repeated syringing through a fine gauge needle. The samples were stored frozen at −70°C until analysed.

(2) As above but the cell pellets were pretreated with ice cold 4.2% w/w perchloric acid (prepared by dilution of perchloric acid, 70% w/w Analar) for 1 min followed by centrifugation of precipitated protein and nucleic acids. The precipitate was washed once with distilled water and then dissolved in lysis buffer.

Samples of c-MYC recombinant protein generously supplied by S. Pennington (Watt et al. 1985) were used as positive controls and in some cases were added to cell pellets of the control lymphocytes immediately prior to lysis as an internal standard to check for breakdown of the protein.

Total protein was assayed using the Micro BCA technique (Pierce Chemical Co.), and fol-

lowing treatment with 2-mercaptoethanol at 100°C for 5 min, known amounts of lysate (as determined by total protein content) were loaded onto each lane of the gels. Prestained molecular weight markers (Bio-Rad Laboratories) were used as molecular weight controls and to check the efficiency of transfer of protein from the gel to nitrocellulose. Detection of the c-MYC protein was with a double antibody technique using an anti c-MYC monoclonal antibody obtained from Oncogene Science, and derived from the 9E10 hybridoma clone (Evan et al., 1985). This antibody was found to be the most sensitive and reliable of a number of commercially available antibodies tested. Blots were developed using an alkaline phosphatase conjugated second antibody (goat anti-mouse, Dakopatts D314) and a chromogen solution consisting of 330 μg/ml of nitroblue tetrazolium and 165 μg/ml 5-bromo-4-chloro-3-indolyl phosphate in a 0.1 M Tris, 0.1 M sodium chloride, 0.1 M magnesium chloride buffer pH 9.5. The lanes were scanned using a Shimadzu flying spot densitometer (Shimadzu Corporation, Japan) and the areas of the c-MYC protein absorbance peaks were recorded.

Results and discussion

The effects of PHA stimulation on c-MYC protein content of human peripheral lymphocytes was investigated in more detail than hitherto reported, in order to establish a well characterized test system for evaluating MYC anti-sense oligonucleotide analogues. Throughout the study, the kinetics of in vitro PHA-induced mitogenesis

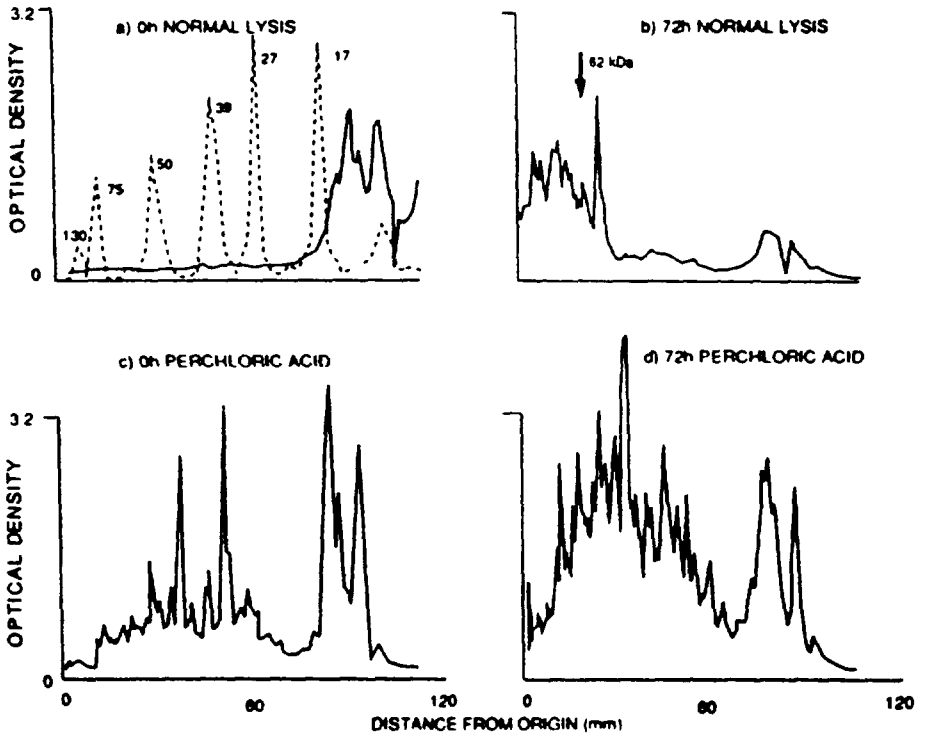

Fig. 1. Densitometry scans of Coomassie blue stained SDS PAGE gels (10% acrylamide). *a*: PHA treated lymphocytes 0 h after the addition of mitogen. The cell extract was prepared using the conventional lysis technique (protocol 1 in the text). The dashed line represents the prestained molecular weight markers and is labelled in kDa. *b*: PHA treated lymphocytes 72 h after the addition of mitogen. The cell extract was prepared using the conventional lysis technique (protocol 1 in the text). The arrow at 62 kDa indicates the position of the c-MYC protein extrapolated from the Western blots. *c*: PHA-treated lymphocytes 0 h after the addition of mitogen. The cell extract was prepared using perchloric acid pretreatment (protocol 2 in the text). *d*: PHA treated lymphocytes 72 h after the addition of mitogen. The cell extract was prepared using perchloric acid pretreatment (protocol 2 in the text).

in human mixed lymphocyte cultures were checked by flow cytometry of propidium iodide stained cell samples. These measurements demonstrated that the proportion of lymphocytes in S, G2 and M phases of the cell cycle increased by 18–24 h after mitogen addition (data not shown), indicating that the lymphocytes were successfully stimulated and that the time course of induction of DNA synthesis in our hands was similar to that previously reported (Kelly et al., 1983). The specificity of the Western blotting technique for detecting 62 kDa c-MYC protein was evaluated using essentially pure recombinant product as a standard. Detection of the c-MYC protein was blocked by absorption of the primary antibody with a peptide epitope, while the goat anti-mouse, secondary antibody on its own gave no detectable signal (data not shown).

At the outset, measurements of cellular c-MYC protein content were made by the Western blotting technique, using cell lysates prepared at intervals after mitogen addition with standard lysis buffers containing protease inhibitors. Under these experimental conditions, and despite the presence of the protease inhibitors, protein was found to be generally degraded in the 0 h lysates (Fig. 1*a*) and early time point samples, but less so in the 72 h cell lysates (Fig. 1*b*). At the same time, c-MYC protein was undetectable in all but the 48 h and 72 h samples, even when it was added in recombinant form as an internal standard (Fig. 2*a*).

Other lysis buffers containing cocktails of protease inhibitors as described by Heikkila et al. (1987), (0.01 M Tris-HCl pH 7.5 containing 0.144 M NaCl, 0.5% NP-40, 0.5% SDS, 0.1% Trasylol, 1 mM PMSF, 10 mM iodoacetamide) and Persson et al. (1984) (0.05 M Tris-HCl pH 7.5 containing 0.15 M NaCl 0.1% SDS, 1% Triton X-100, 0.5% sodium deoxycholate, 1 mM PMSF) also did not prevent general protein degradation in lymphocyte lysates. These results were highly unusual since no proteolysis at all was observed, and c-MYC protein was easily detectable, when the same lysis buffers were used to prepare extracts of a range of established human cell culture lines including HL60, Raji, MOLT-4, and other lymphocytic lines. Addition of recombinant c-MYC protein to cell pellets, prior to lysis, demonstrated

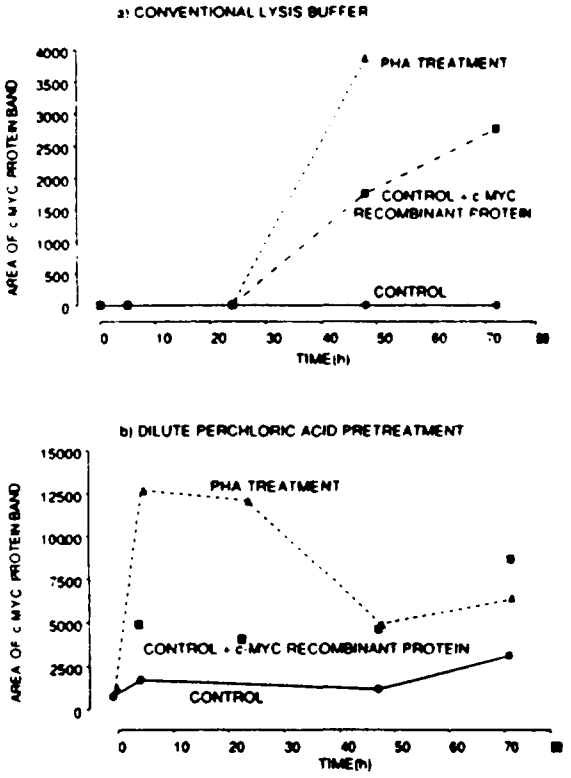

Fig. 2. Variation of c-MYC protein content of lymphocyte lysates with time after PHA stimulation. Western blots were scanned with a densitometer and the area of the c-MYC protein band was plotted against the time after PHA addition. Circles represent control samples with no mitogen added, triangles represent PHA treated samples, and squares represent control samples to which recombinant c-MYC protein was added. *a*: Concentration of c-MYC protein in lymphocyte lysates prepared using the conventional lysis technique (protocol 1 in the text). *b*: Concentration of c-MYC protein in lymphocyte lysates prepared using perchloric acid precipitation (protocol 2 in the text).

that the protease inhibitor-resistant proteolytic activity in human peripheral lymphocytes decayed, even in the absence of mitogen, during incubation in tissue culture medium at 37°C (Fig. 2*a*).

It was apparent that an alternative to the standard protease inhibitor cocktails was required to rapidly inactivate the protease(s) of human peripheral lymphocytes if their c-MYC protein content were to be measured. Heat treatment was tested as an initial approach to resolving this problem. However, although boiling in-

mediately after lysis prevented most of the protein degradation at early time points, added c-MYC protein was still degraded (results not shown), suggesting that proteolysis was very rapid. Another approach, which did prove to be successful, was that of dilute perchloric acid fixation. Dilute perchloric acid (4.2% w/w) is routinely used for precipitating cellular macromolecules and extracting acid-soluble monomers in quantitation of cellular nucleotide pools (e.g., Tidd and Paterson, 1974), and at this concentration does not cause significant chemical modification, other

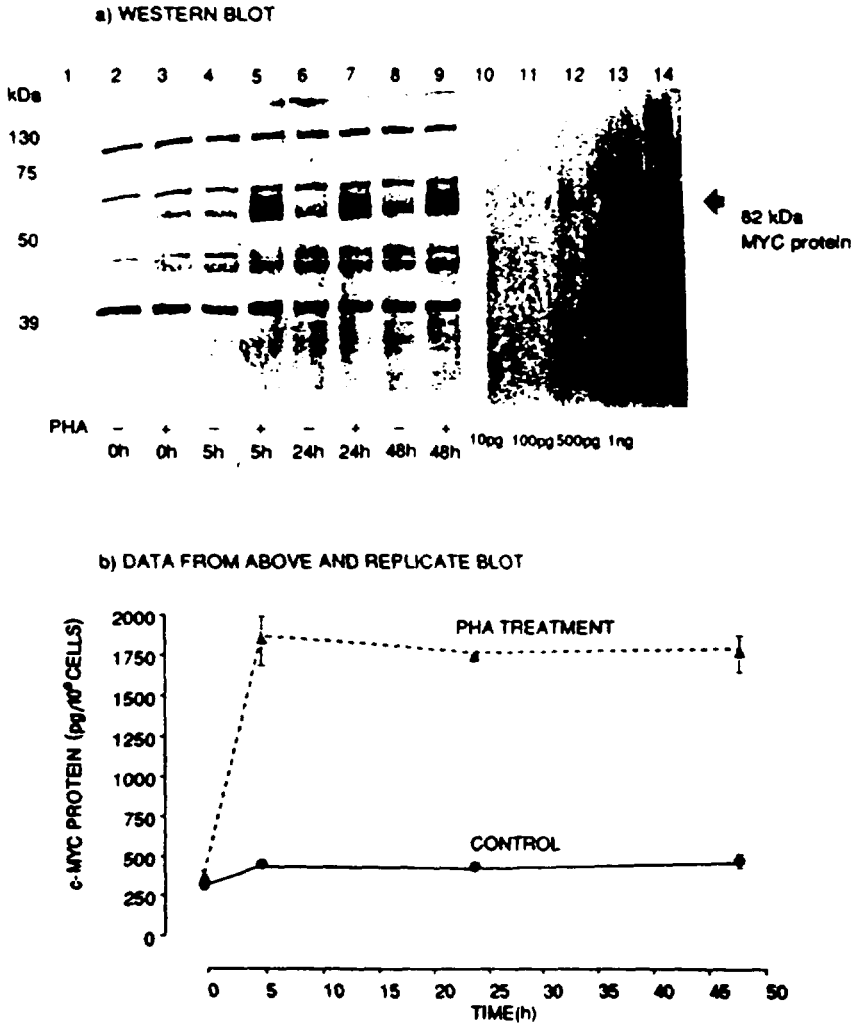

Fig. 3. Variation of c-MYC protein concentration in lymphocytes with time after PHA stimulation. *a*: an example of the Western blots achieved using perchloric acid pretreatment. Lane 1, prestained molecular weight markers; lane 2, no treatment 0 h; lane 3, PHA treatment 0 h; lane 4, no treatment 5 h; lane 5, PHA treatment 5 h; lane 6, no treatment 24 h; lane 7, PHA treatment 24 h; lane 8, no treatment 48 h; lane 9, PHA treatment 48 h; lanes 10–13, 10 pg, 100 pg, 500 pg, 1 ng of c-MYC recombinant protein; lane 14, prestained molecular weight markers. *b*: densitometric analysis of the Western blot illustrated in *a*, and a replicate blot. The error bars indicate the standard deviation where this is larger than the symbol. The concentration of c-MYC protein is expressed as pg per 10^6 cells, and was derived from the c-MYC recombinant protein standard curve.

than denaturation of cellular constituents. Although at higher concentrations perchloric acid is a strong oxidising agent, at the low concentration specified perchloric acid failed to oxidise the highly susceptible 6-thiopurines (Doerr et al., 1961; Tidd and Dedhar, 1978) and therefore, it is unlikely that significantly more epitope destruction would occur using perchloric acid than already prevails under the denaturing and reducing conditions of the SDS-PAGE Western blotting procedure. At the same time, similar results to those described below might be achieved by using non-oxidising protein precipitants such as trichloroacetic acid, although this was not tested. Following perchloric acid fixation, the denatured macromolecular pellet may be dissolved in standard cell lysis buffer and may still be used in Western blotting analysis, since the technique requires that the proteins be denatured. When dilute perchloric acid fixation was applied to in vitro incubated human lymphocyte cell pellets, prior to their dissolution in lysis buffer, c-MYC protein was detectable by Western blotting analysis in all samples, including 0 h and untreated control cell lysates (Figs. 2 and 3), and many more high molecular weight proteins were observed (Figs. 1*c* and 1*d*). Experiments conducted using this lysis procedure demonstrated that large increases in cell content of c-MYC protein occurred within 5 h of PHA stimulation (Fig. 2*b*). Though some variation in lymphocyte response to PHA at later times was observed between different blood donations (for example see the difference at 48 h between Fig. 2*b* and Fig. 3*b*).

The signal from c-MYC protein internal standard added to pellets of resting lymphocytes immediately prior to perchloric acid precipitation was similar to the same amount analysed directly (for example the average percentage recovery for the experiment illustrated in Fig. 2*b* was 72% ± 11.5%) indicating that perchloric acid pretreatment did not interfere with c-MYC protein detection. These results established unequivocally that proteolysis and not the limit of sensitivity of the Western blotting system was responsible for the inability to detect c-MYC protein when the perchloric acid precipitation step was omitted (Fig. 2). Moreover, significant concentrations of c-MYC protein were detected in unstimulated lymphocytes when perchloric acid pretreatment was employed (Figs. 2 and 3), whereas previously the protein had been undetectable (Heikkila et al., 1987). Presumably, the concentration of c-MYC protein in resting lymphocytes is below a threshold value, and only above this is it able to function in its putative role as a signal transduction molecule for triggering cell proliferation.

The Western blotting system was calibrated by running various amounts of recombinant c-MYC protein on the gels. The detection limit of the system was found to be approximately 100 pg of the standard (Fig. 3*a*). The amount of c-MYC protein per 10^6 cells was calculated from the calibration curve. An example of the quantitative results obtained from experiments where lymphocyte cell pellets were pretreated with perchloric acid is presented in Fig. 3, which shows that an approximately six-fold increase in mean intracellular c-MYC protein concentration occurred within 5 h of PHA stimulation.

Mention should be made of the fact that the 9E10 antibody used in Western blot development not only bound the 62 kDa c-MYC nuclear protein, but also detected other proteins (Fig. 3*a*), as did all the other antibodies tested. The spurious signals were different for each antibody but could be reduced in intensity relative to the c-MYC band (at least in Colo 320 and HT29 cell lysates) if nuclear fractions were used as opposed to whole cell lysates (data not shown). The demonstration of additional interactions has serious implications if these antibodies are to be used for immunohistochemical or flow cytometric analysis in which no check of the molecular weights of the antigens is possible, and results from such techniques must be interpreted with caution.

Further studies comparing perchloric acid pretreatment to conventional cell lysis are required in order to determine whether the agents responsible for the observed protein degradation are limited to lymphocytes or are present in other tissues. One area where problems may occur is in measuring c-MYC protein in tumour biopsies since lymphocytes can be a significant contaminant. In addition, the measurement of other proteins in human peripheral lymphocytes might benefit from perchloric acid pretreatment since general protein degradation was observed when

this step was omitted (Fig. 1), and its use obviates the need for expensive and toxic protease inhibitors.

In conclusion, use of the modified cell lysis procedure described in this note resulted in the novel observation that resting human lymphocytes contain significant concentrations of c-MYC protein. PHA stimulation induced increases in cell content of the oncogene protein which paralleled the reported increase in c-MYC mRNA during lymphocyte mitogenesis (Kelly, 1983).

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Short Term Administration of Potassium Perchlorate Restores Euthyroidism in Amiodarone Iodine-Induced Hypothyroidism*

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ABSTRACT. We studied the effect of potassium perchlorate ($KClO_4$) in patients with hypothyroidism due to amiodarone. The short term administration of $KClO_4$ to six such patients led to prompt restoration of euthyroidism, while the three untreated patients remained hypothyroid for 2–6 months. Since $KClO_4$ inhibits thyroid iodide transport, thereby blocking further entrance of iodide into the thyroid and decreasing intrathyroidal iodide content, amiodarone-associated hypothyroidism is probably secondary to the inhibitory effect of excess intrathyroidal iodine on thyroid hormone synthesis. (*J Clin Endocrinol Metab* 63: 1233, 1986)

AMIODARONE, a drug containing 37.2 mg iodine/100 mg active substance, is widely used for the treatment of cardiac tachyarrhythmias. This agent regularly alters serum thyroid hormone concentrations and may cause iodine-induced hyperthyroidism or hypothyroidism (1–17), since free iodine is released from the metabolism of the drug *in vivo* (18). Hypothyroidism may develop in patients with or without underlying thyroid disorders (7–11, 16, 17). The mechanism responsible for thyroid insufficiency in amiodarone-induced hypothyroidism is not certain, although it is probably related to the inhibitory effect of excess intrathyroidal iodide on thyroid hormone synthesis (7, 18, 19). We recently reported that the short term oral administration of potassium perchlorate ($KClO_4$) together with methimazole led to a rapid control of amiodarone iodine-induced hyperthyroidism, probably through intrathyroidal iodide depletion by $KClO_4$ (20). In the present study, we assessed the effects of $KClO_4$ administration in patients with amiodarone-induced hypothyroidism.

Subjects and Methods

Patients

Nine patients treated with chronic amiodarone therapy for various cardiac tachyarrhythmias (500–750 mg/week) for a period of 9–36 months developed clinical and biochemical evidence of hypothyroidism. The relevant clinical and biochemical features of the hypothyroid patients at the time of entry into the study are summarized in Table 1. Amiodarone was discontinued in all patients at this time. Patients 2 and 4 had no evidence of underlying thyroid disease, as assessed by physical examination and ultrasound; four patients had Hashimoto's thyroiditis; one had a small goiter; one had been treated previously with ^{131}I for hyperthyroidism due to Graves' disease; and the final patient had received x-ray treatment to the neck for laryngeal cancer 6 yr previously. After withdrawal of amiodarone, patients 1, 2, and 3 were followed for 80, 300, and 155 days, respectively, without treatment, while patients 4–9 received a single dose of 1.0 g $KClO_4$ daily for 9–34 days and then were followed for an additional 60–228 days, except for patient 9 who was followed for only 4 days after stopping $KClO_4$. During $KClO_4$ administration, the patients received a β-adrenergic antagonist drug or a calcium channel-blocking drug as needed for their cardiac problems.

Laboratory tests

Serum total T_4 and total T_3 concentrations were measured by RIA using commercial kits (ARIA II, Becton Dickinson Laboratory, Milan, Italy). T_3 resin uptake was measured using Trilute Kits (Miles S.p.A., Ames Division, Milan, Italy). Free T_4 (FT_4I) and T_3 (FT_3I) indices were calculated as the product of the T_3 resin uptake and total serum T_4 and T_3 concentrations, respectively. Since the changes in the serum total thyroid hormone concentrations were similar to those in the free thyroid hormone indices, only FT_4I and FT_3I values are reported. Serum TSH was measured by an ultrasensitive TSH immuno-

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TABLE 1. Clinical and biochemical features in patients with amiodarone iodine-induced hypothyroidism

Patient No.	Sex	Age (yr)	Drug Rx (months)	FT_4I	FT_3I	TSH (μU/ml)	Ab-M* titer	Thyroid 24-h ^{131}I uptake (%)	Thyroid disease
Amiodarone withdrawal group									
1	F	55	12	<2	<50	80	1:102,400	2	Hashimoto's thyroiditis
2	M	54	36	2.5	121	66	Negative	9	None
3	M	76	13	<2	<50	208	1:1,600	25	Hashimoto's thyroiditis
$KClO_4$ administration group									
4	M	82	24	<2	71	131	Negative	3	None
5	M	75	12	<2	<50	260	Negative	3	Previous neck irradiation
6	M	49	9	3.7	71	101	Negative	9	Graves' disease (after ^{131}I Rx)
7	F	56	24	2.3	90	100	1:102,400	19	Hashimoto's thyroiditis
8	F	63	22	3.7	134	35	Negative	10	Small goiter
9	F	35	10	3.8	157	23	1:25,600	10	Hashimoto's thyroiditis
Normal values				4.1-12	100-206	0.5-4.6	≤1:400	22-48	

* Ab-M, Antithyroid microsomal antibody.

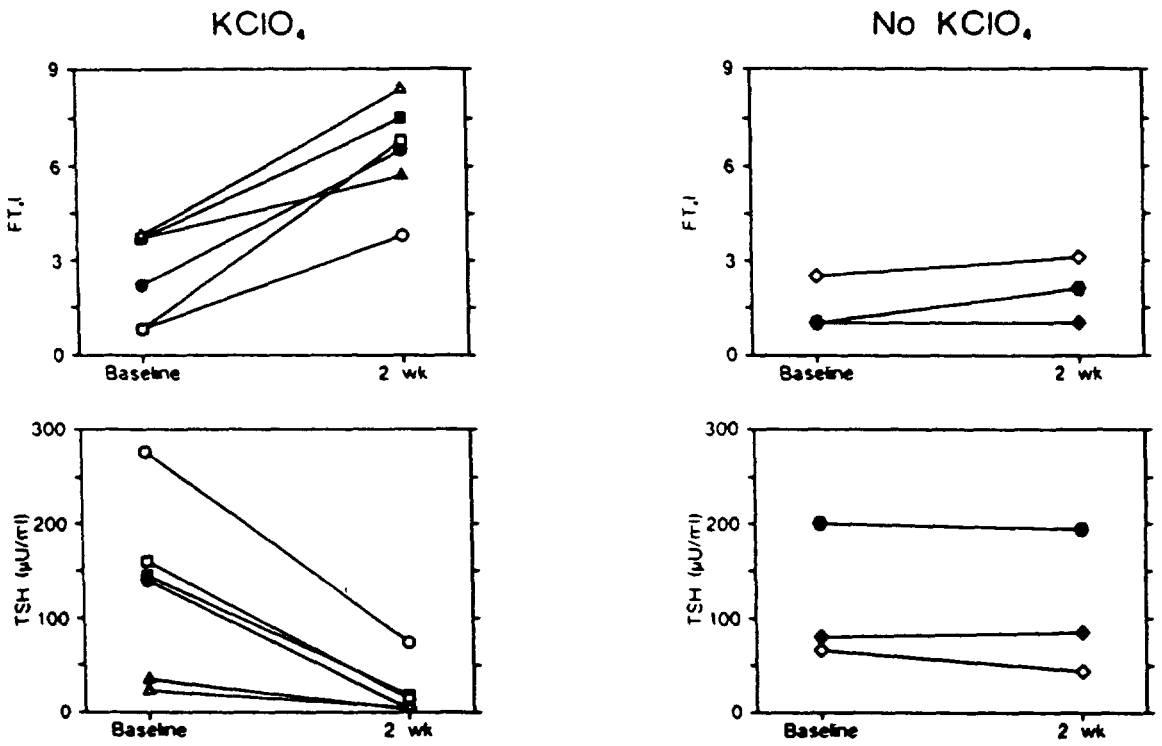

FIG. 1. Serum FT_4I values and TSH concentrations in hypothyroid patients. Amiodarone was discontinued at the time of the baseline value. Patients were then treated with $KClO_4$ or were followed without treatment, and blood was obtained 2 weeks later. Each symbol represents an individual patient: ♦, 1; ◇, 2; ●, 3; ●, 4; ○, 5; ■, 6; □, 7; ▲, 8; △, 9.

radiometric assay (Boots-Celltech, Nottingham, United Kingdom). Antithyroid microsomal autoantibodies were measured by passive hemagglutination (Fujizoki, Tokyo, Japan). ^{131}I Thyroid uptake was measured at 24 h.

Results

$KClO_4$ administration increased serum FT_4I values and decreased serum TSH concentrations. In five of the patients who received $KClO_4$, serum FT_4I increased to normal within 2 weeks after initiation of $KClO_4$ administration (Fig. 1). Serum TSH concentrations decreased markedly in all patients who received $KClO_4$ and were normal in three of the six patients 2 weeks after starting $KClO_4$ treatment. Serum FT_4I values and TSH concentrations changed very little in the three patients who did not receive $KClO_4$.

After withdrawal of $KClO_4$, hypothyroidism recurred in three of the six patients. Patients 5, 6, and 7 became hypothyroid within 5, 73, and 47 days, respectively, after discontinuing $KClO_4$. The results in patient 7, who received $KClO_4$ twice, are shown in Fig. 2. On both occasions, treatment with $KClO_4$ was associated with a rapid

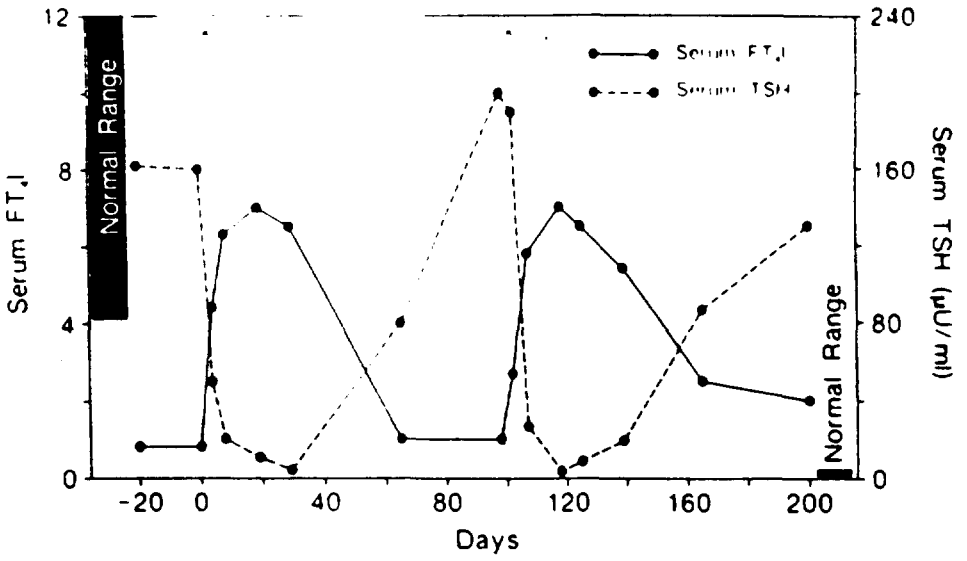

FIG. 2. Serum FT_4I values and TSH concentrations in patient 7, who received $KClO_4$ on two occasions.

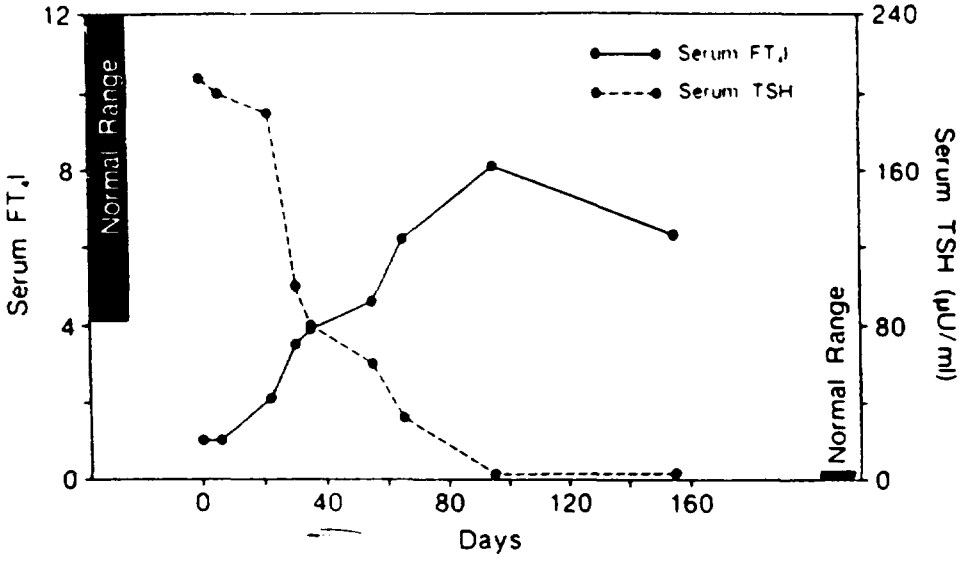

FIG. 3. Serum FT_4I values and TSH concentrations in patient 3, who did not receive $KClO_4$.

increase in serum FT_4I values and a rapid decrease in serum TSH concentrations, followed by relapse when $KClO_4$ was discontinued. Patients 4 and 8 remained clinically euthyroid and had serum FT_4I values of 7.6 and 6.4 and serum TSH levels of 1.3 and 1.8 μU/ml when last studied 8 and 2 months, respectively, after withdrawal of $KClO_4$. Patient 9 was not followed long enough to determine her ultimate thyroid status.

Two of the three patients (patients 2 and 3) who did not receive $KClO_4$ became euthyroid after 180 and 95 days, respectively. The data for patient 3 are shown in Fig. 3. Patient 1 remained profoundly hypothyroid for over 80 days (serum FT_4I, 3.0; TSH, >80 μU/ml) and, therefore, was treated with L-T_4.

No side effects or toxic reactions occurred during $KClO_4$ therapy, as assessed by hematological, hepatic, and renal function tests.

Discussion

Long term treatment with amiodarone may induce hypothyroidism in patients with or without underlying thyroid disorders (7–11, 16, 17), and amiodarone-induced hypothyroidism is more common in areas with sufficient iodine intake, especially in patients with Hashimoto's thyroiditis (7, 8) who are more susceptible to iodine-induced hypothyroidism (21). The development of amiodarone-induced hypothyroidism appears to be the consequence of the inhibitory effect of excess intrathyroidal iodide on the synthesis of the thyroid hormones (19). While patients with underlying defects in the intrathyroid organification of iodine are more susceptible to the inhibitory effects of excess iodine, high intrathyroidal iodine concentrations may induce decreased hormone synthesis in patients with apparently normal thyroid

glands. Thus, Hawthorne *et al.* (17) demonstrated positive perchlorate discharge tests in seven of eight patients with amiodarone-induced hypothyroidism, six of whom had no evidence of previous thyroid disease. One of them was restudied 9 months after stopping amiodarone and was found to have a normal perchlorate discharge test.

In the present study, we evaluated the possibility of restoring the euthyroid state by administrating $KClO_4$. This anion reduces intrathyroidal iodide content by competitively inhibiting thyroid iodide transport, so that sufficient iodide to inhibit thyroid hormone synthesis is no longer present within the thyroid follicular cells. The results obtained confirm the role of intrathyroidal iodine in the genesis of hypothyroidism in amiodarone-treated patients, since a prompt rise in circulating thyroid hormone and a decrease in serum TSH concentrations occurred during $KClO_4$ administration.

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Two cases of amiodarone-induced thyrotoxicosis successfully treated with a short course of antithyroid drugs while amiodarone was continued

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Abstract

Two patients with amiodarone-induced thyrotoxicosis were treated successfully with potassium perchlorate and carbimazole while treatment with amiodarone was continued. These antithyroid drugs were stopped after the patients had became clinically and biochemically euthyroid. During follow up, when treatment with amiodarone continued, thyrotoxicosis did not recur.

Amiodarone-induced thyrotoxicosis seems to be a transient condition that can be treated successfully with a short course of antithyroid drugs without stopping amiodarone treatment.

(*Br Heart J* 1994;72:266–268)

Amiodarone is an effective antiarrhythmic agent with a high iodine content that affects the production and secretion of thyroid hormones and in some patients induces overt thyrotoxicosis or hypothyroidism. The mechanism responsible is incompletely understood. An iodine excess is not the prime determinant.[1] Amiodarone could induce hypothyroidism by unmasking a pre-existing subclinical abnormality.

Amiodarone induced thyrotoxicosis is unpredictable with an unexplained sudden onset in patients with previously normal thyroid function and without pre-existing thyroid antibodies. The chief hypothesis for the pathogenesis of amiodarone induced thyrotoxicosis is that amiodarone or its metabolites damage the thyroid cells and disrupt follicles, resulting in the release of thyroid hormones into the circulation.[2] This hypothesis implies that treatment with amiodarone must be stopped. This may result in the recurrence of the life-threatening tachycardia. Martino *et al* reported that stopping amiodarone treatment and starting treatment with potassium perchlorate and methimazole led to a resolution of thyrotoxicosis within 16–36 days.[3] Reichert *et al* reported the successful treatment of amiodarone induced thyrotoxicosis with potassium perchlorate and methimazole, while treatment with amiodarone was continued, in three patients.[4] These patients became euthyroid in 2–5 weeks. Then they were treated with a combination of amiodarone and methimazole.

We report two patients in whom amiodarone induced thyrotoxicosis was successfully treated with potassium perchlorate and carbimazole while treatment with amiodarone was continued.

Case reports

CASE 1 (TABLE 1)

A man aged 18 presented in 1985 with ventricular flutter caused by arrhythmogenic right ventricular dysplasia. The tachycardia did not respond to several antiarrhythmic drugs. Treatment with amiodarone (400 mg/day) was started in 1988. There were no further recurrences of tachycardia. Before treatment with amiodarone was started the patient was clinically euthyroid, with no goitre and concentrations of thyroid hormones within normal range and no thyroid autoantibodies.

Two years later he attended the outpatient

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Table 1 Clinical state and hormone concentrations in patient 1

Time (mth)	*0*	*15*	*28*	*31*	*33*	*35*	*37*	*55*
Clinic	EU	EU	EU goitre	EU BH	AIT	EU	EU TSH normal	EU VT
Treatment	start A				start C+K	C+K	C+K stop	
Serum amiodarone mmol/l	-	0·70	1·2		1·5		0·9	0·2
Serum desethylamiodarone mmol/l	-	0·45	0·85		0·7		0·9	0·4
TSH mU/l	5·4	1·8	3·6	<0·1	<0·1	<0·1	1·6	6·0
T4 (nmol/l)	95	160	145	215	260	170	110	115
FT4 (pmol/l)	13·3	20·8	21·0		59·8	25·5	17·0	18·4
T3 (nmol/l)	1·5·0	1·75	1·55		3·10	2·30	1·00	1·35
rT3 (nmol/l)	0·24	0·75	0·77		1·10	0·84	0·56	0·35
TG (ng/ml)	32	175	380		1700	820	200	110
TgAb McAb					+/+		+/+	

AIT, amiodarone induced thyrotoxicosis; C, carbimazol; EU, euthyroid (clinical); FT4, free T4; K, potassium perchlorate; McAb, microsomal antibodies; rT3, reverse T3; T3, triiodothyronine; T4, thyroxine; TgAb, thyroglobuline antibodies; TSH, thyroid stimulating hormone

Table 2 Clinical state and hormone concentrations in patient 2

Time (mth)	*0*	*1*	*2*	*3*	*40*	*44*	*49*
Clinic	EU	EU	AIT	AIT	EU	EU TSH normal	EU
Treatment	start A			start C + K	C + K	C + K stop	
Serum amiodarone (mmol/l)		2·1		2·0	2·7	3·0	3·2
Serum desethylamiodarone		0·67		2·01	2·5	2·2	2·4
TSH (mu/l)	0	1·5	<0·1	<0·1	<0·1	0·1	3·1
T4 (nmol/l)	115	191	99	310	290	270	265
FT4 (pmol/l)		33	23	51·1	37·7	29·7	29·2
T3 (nmol/l)	1·75	1·45	2·05		2·00	1·50	1·60
rT3 (nmol/l)	0·11	0·80	1·56	1·08	1·12	0·77	0·72
TG (ng/ml)	13	9	130	55	38	44	95
TgAb McAb						- -	

AIT, amiodarone induced thyrotoxicosis; C, carbimazol; EU, euthyroid clinical; FT4, free T4; K, potassium perchlorate; McAb, microsomal antibodies; rT3, reverse T3; T3, triiodothyronine; T4, thyroxine; TgAb, thyroglobuline antibodies; TSH, thyroid stimulating hormone

department complaining of dysphagia. The physical examination showed no evidence of hypothyroidism but a tender diffuse goitre was palpable. Thyroid hormone concentrations were within the normal range, but the concentration of thyroglobulin was high because of the goitre. Two months later TSH was abnormally low and thyroxine was abnormally high, although the patient still showed no clinical signs of hyperthyroidism.

Two months later, when the cumulative dose of amiodarone was 40 g, clinical overt thyrotoxicosis suddenly developed: the tender goitre was still present. Thyroid stimulating hormone (TSH) was undetectable; thyroxine (T4), free thyroxine (FT4), and triiodothyronine (T3) were raised; the thyroglobulin concentration had increased further. Concentrations of amiodarone and desethylamiodarone were in the therapeutic range (table 1). Because the erythrocyte sedimentation rate was normal and because the goitre had been present for more than two months we excluded the diagnosis of subacute thyroiditis of the Guervain type and diagnosed amiodarone induced thyrotoxicosis.

Treatment with carbimazole (40 mg/day) and potassium perchlorate (1 g/day) was started and treatment with amiodarone was continued. Clinical euthyroidism was achieved after 2 months. TSH was still undetectable, but T4 and T3 concentrations were almost normal. The TSH concentration became normal after 4 months. The thyroid gland was still slightly enlarged but no longer painful. The antithyroid medication was stopped. Although treatment with amiodarone continued clinical and biochemical thyroid dysfunction did not recur in the next 18 months. The goitre was no longer palpable. Ventricular tachycardia recurred and amiodarone and desethylamiodarone concentrations were below the therapeutic range of 0·2 and 0·4 mmol/l respectively. This confirmed our suspicion that the patient had stopped taking amiodarone between week 40–45 of the follow up.

CASE 2 (TABLE 2)

A man born in 1936 had an inferolateral and right ventricular myocardial infarction complicated by cardiac aneurysm, severe mitral valve regurgitation, and poor left ventricular function. Late ventricular tachycardia was treated with several antiarrhythmic drugs (flecainide, chinidin, disopyramide) without success until amiodarone was started (800 mg/day). Before treatment with amiodarone the patient was clinically and biochemically euthyroid. When the dose of amiodarone was reduced to 200 mg ventricular tachycardia recurred; a daily dose of 400 mg prevented tachycardia.

After 37 months, when the cumulative amiodarone dose was 44 g, overt amiodarone induced thyrotoxicosis developed. Serum T4 and FT4 concentrations were extremely high. One week later thyroxine and triiodothyronine concentrations had already fallen slightly. Treatment with carbimazole (40 mg/day) and potassium perchlorate (1 g/day) was started, while amiodarone medication was continued. The patient became clinically euthyroid after two months. When the thyroid stimulating hormone concentration became normal six months later, the anti-thyroid medication was stopped. Though he continued to take amiodarone, confirmed by measuring the serum concentrations of amiodarone and desethylamiodarone, thyroid dysfunction did not recur during the follow up of seven months. The patient died suddenly at home.

Discussion

Amiodarone induced thyrotoxicosis in these two patients was transient. It was successfully treated with a short course of antithyroid drugs while amiodarone was continued. When amiodarone is stopped because of amiodarone induced thyrotoxicosis life threatening tachycardia can recur. Under these circumstances total thyroidectomy has been advocated to allow treatment with amiodarone to continue.[5] It is remarkable that a short course of antithyroid drugs restored euthyroidism, and that the patients remained euthyroid despite continuing treatment with amiodarone.

The mechanism of effects of amiodarone on the thyroid is multifactorial and largely

unknown. Although auto-immune related thyrotoxicosis has been reported after amiodarone treatment,[6] neither of our patients had a goiter or thyroid antibodies before amiodarone treatment and antibodies did not develop during follow up.

These data suggest that amiodarone induced thyrotoxicosis has a peculiar pathogenesis. When intrathyroidal amiodarone concentrations exceed a threshold, cell damage leads to thyrotoxicosis when the contents of the thyroid leak into the bloodstream. The intra-thyroidal concentration of amiodarone too would decrease, allowing repair and the restoration of euthyroidism. If this hypothesis is true, continuation of amiodarone treatment might eventually lead to a recurrence of thyrotoxicosis when the intrathyroidal amoidarone concentration again exceeds the threshold. In our patients the follow up period was probably too short for a second period of thyrotoxicosis to develop.

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CORRECTION

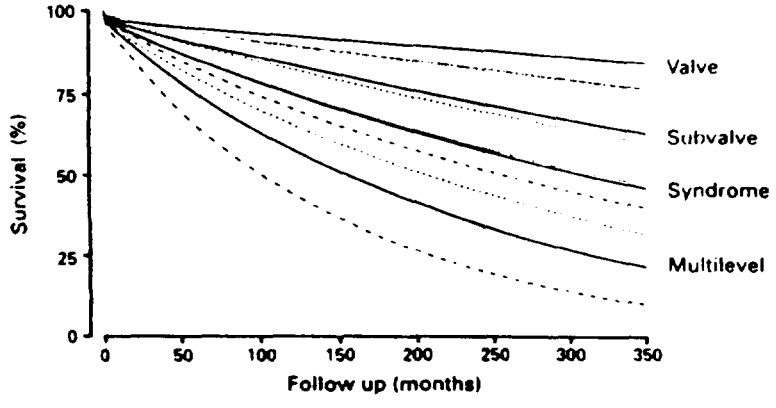

Figure 1 Predicted risk of death (and 70% CIs) for a patient presenting at 13·9 months of age with moderate obstruction of the left ventricular outflow tract plotted against level of obstruction from a solution to equations developed by means of hazard analysis (appendix 1).

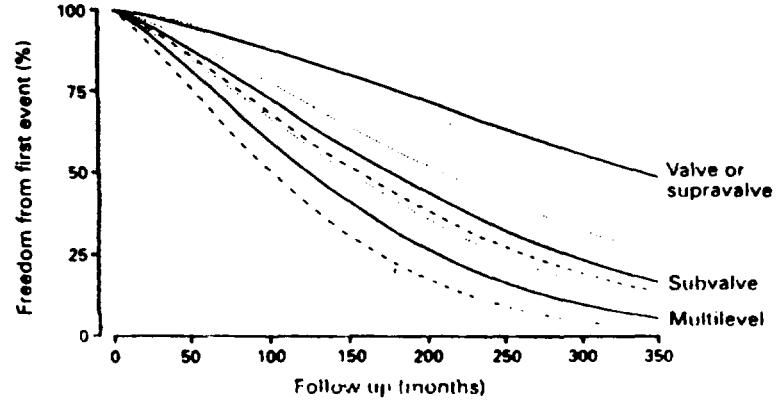

Figure 2 Predicted freedom from first clinical event (operation, balloon dilatation or endocarditis, and 70% CIs) for a patient presenting at 13·9 months of age with mild obstruction of left ventricular outflow tract without aortic regurgitation plotted against level of obstruction from equations developed by means of hazard analysis (appendix 1).

Incidence and prognosis of obstruction of the left ventricular outflow tract in Liverpool (1960–91): a study of 313 patients

D Kitchener, M Jackson, N Malaiya, K Walsh, I Peart, R Arnold

We regret that owing to a printer's error figure 1 and figure 2 in this article in the June issue (*Br Heart J* 1994;71:588–95) appeared in the wrong order and with the wrong legends. The corrected versions are reprinted on the left.

not able to expectorate his sputum due to muscle weakness, a Cook minitracheostomy was introduced in order to allow endotracheal suctioning. On the 5th day after minitrach insertion the cannula dislodged from its flanges and was inhaled. On a chest X-ray it was located in the left mainstem bronchus (Fig. 1). The patient was reintubated and the cannula was removed by rigid bronchoscopy. Following this episode the patient experienced another myocardial infarction, accompanied by left ventricular and respiratory failure. In face of the poor prognosis, further treatment was discontinued and the patient died two days later.

The Seldinger technique is theoretically a useful method for introducing a minitracheostomy. A 'fausse' route is less likely to occur, and bleeding is tamponaded directly by the dilators. The material of the Cook minitracheostomy is softer than the Portex and is less likely to cause damage to the subglottic region.

This case however, shows that the cannula could losen from its flanges. The reason for this is unknown, since another minitracheostomy cannula could not be separated by pulling forcefully. It is possible that saliva and sputum changed its consistency and loosened the fixation with which the cannula is clasped to its flanges.

We feel that the design of this cannula needs further investigation. In the meanwhile Cook has withdrawn this type of minitracheostomy from the market.

Yours faithfully

J.C.M. Beenakkers and Ch.P. Stoutenbeek

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Methaemoglobinaemia: an unusual case report

Dear Sir,

We should like to report an unusual case of methaemoglobinaemia.

A 42-year-old man was involved in an accidental shell blast. We admitted a conscious patient, with blood pressure 150/80 mmHg, heart rate 90/min and spontaneous breathing (oxygen concentration 40%). He showed a complete left forearm section, burns of the left orbital region (area 5%) and of the low limb (area 20%) associated with a thigh wound caused by a shell-splinter. Under general anesthesia, the left forearm was amputated. Meanwhile, despite normals blood gases analysis (pH 7.39, PaO_2 19.8 kPa, $PaCO_2$ 5.1 kPa, SaO_2 – calculated – 99.3%) the blood colour remained dark. A gradual FIO_2 increase (from 0.40 to 0.65) and a positive end-expiratory pressure were without any effect.

At day 1, despite a normal haemoglobinaemia (13.9 g/dl) and a satisfactory blood gas analysis (pH 7.38, PaO_2 14.1 kPa, $PaCO_2$ 5.6 kPa, SaO_2 99.7%), we noticed a brown-red coloured blood during arterial punctures and cyanosis of the extremities. Biochemical investigations showed: carboxyhaemoglobinaemia: 0% (normal value: 0% to 3%), methaemoglobinaemia: 28.7% (normal value less then 0.8%).

The mine clearance departement communicated to us the results of the bomb contents: a 54 kg German shell, manufactured in 1916, that contained 14 kg explosives: 60% potassium perchlorate (8 kg), 40% dinitrobenzene (6 kg) and a small amount of picric acid (0.1 kg) and melinite (0.1 kg). We diagnosed a moderate methaemoglobinaemia (28.7%) due to dinitrobenzene and/or potassium perchlorate.

The patient was treated with intravenous ascorbic acid (1000 mg) and a 5 h continuous infusion of methylene blue (150 mg in 250 ml 5% glucose solution).

Toxic methaemoglobinaemia is due to oxyhaemoglobin oxidation, usually caused by chemicals or drugs. Our report of methaemoglobinaemia following a shellblast is more uncommon, and two chemicals may have been involved potassium perchlorate and dinitrobenzene. Potassium perchlorate gives methaemoglobinaemia when it is ingested and this cannot be evoked in our case report. Here, the most probable mechanism was absorption after cutaneous spraying, which has already been described in the literature [3, 5].

The diagnosis of methaemoglobinaemia should be suspected on clinical signs. The major sign is cyanosis, predominant on extremities and lips. The blood is "chocolate-brown" coloured, contrasting with normal gas analysis. Others signs are vertigo, nausea, vomiting when the methaemoglobinaemia is between 30% and 50%. Tachypnoea and cardiac signs exist when the amount is about 70% [2]. The major problem it to evoke the diagnosis. It may be simple when clinical signs are obvious or when the history suggests an intoxication. Otherwise, it is more difficult: either clinical signs are poor, like in our case, or the situation has only been recently described, like methaemoglobinamia following smoke inhalation [1, 4]. One must keep in mind the paradoxical situation of a "chocolate-brown" coloured blood with cyanosis and normal arterial blood gases.

Methaemoglobinaemia should be quantified in every case of a war device explosion if there are clinical signs were may suggest this pathology.

Yours faithfully,
P. Laure and F. Stierle

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Final
2/26/96

Thyroid Hormone and Iodine Requirements in Man during Brain Development

D.A. Fisher, M.D.; F.M. Delange, M.D.

to Joan Dollarhide –
This is a Monograph paper in press outlining infant thyroid iodine & thyroid hormone metabolism. – The perchlorate risk would presumably parallel the iodine "requirement" in infancy vs adults eg 7-15/1 (Table 5). Those infants have very limited iodine / thyroid hormone reserve + actively pump iodine (into follicular cells).

Dfisher

Table of Contents

Thyroid Hormones and Iodine Requirements in Man during Brain Development

Introduction

The dependence of human brain maturation on thyroid hormone has long been recognized. The "critical period" for the dependency is known to extend from birth to 3 years of age with a progressively decreasing dependence over that interval.[1-3] Considerable information has accumulated during the last 20 year era of newborn thyroid screening to quantify the thyroid hormone requirement during this period. Recent evidence in rats and accumulating data from geographic areas of endemic iodine deficiency suggest that the critical period of dependency of brain maturation on thyroid hormones extends to intrauterine life.[4-8] There is limited data to characterize the timing of this intrauterine dependency or quantify the thyroid requirements. The goal of this report is to review the available information defining the thyroid hormone and iodine requirement during the period of human brain maturation.

Thyroid Hormone Metabolism and Requirements during Fetal Life

Thyroid function in the human fetus becomes manifest near the end of the first trimester of gestation.[9,10] Embryogenesis of the hypothalamus, the pituitary and the thyroid gland are largely complete at this time and the thyroid follicular cells are capable of iodothyronine synthesis. The system, however, remains relatively inactive until midgestation; only low levels of TSH and T4 are measurable in fetal serum until 18-20 weeks after which time there is a progressive increase in serum TSH, TBG, total T4 and free T4 levels, all of which plateau at 35-37 weeks. During the latter half of gestation thyroid system development involves complex interactive maturation of hypothalamic-pituitary-thyroid control, peripheral iodothyronine metabolism, and thyroid hormone action at the cellular level.

There is no information regarding the timing of thyroid hormone dependence for various organ systems or cellular maturational events in the human fetus and no quantitative data regarding the maturation of fetal thyroid hormone production or utilization. Qualitative and quantitative information have been developed in the fetal sheep in which species thyroid system maturation closely resembles that in the human fetus.[9-12] The timing is compressed to a 150 day gestation period whereas gestation in the human species continues for 40 weeks or 280 days. The final period of thyroid system maturation in the sheep fetus occurs over a period of 70-80 days, and the last third of gestation in this species covers a time interval of 50 days.

Prominent features of the maturing thyroid metabolic system in the human and sheep species include:

1. Limited permeability of the placenta to thyroid hormones. There are marked transplacental gradients of thyroid hormones at all stages of fetal maturation, and fetal thyroidectomy markedly reduces fetal serum T4 levels during the latter half of gestation.

2. Extrahypothalamic production of TRH. This system may function as an auxiliary hypothalamus during early thyroid system ontogenesis.

3. Fetal production of inactive thyroid hormone analogues. This may be important to minimize fetal catabolism and enhance anabolism in most fetal tissues.

4. Mechanisms for supply of active hormone to selected fetal tissues. These tissues are characterized by the early appearance of T3 nuclear receptors and the Type II MDI to provide preferential local T3 production.

5. Programmed activation of thyroid hormone responsiveness in individual tissues.

This system has been characterized as pluralistic.[11] Each tissue or organ is relatively unique with regard to its role in thyroid metabolism and its dependence and timing of appearance of dependence on thyroid hormones. Most tissues are protected in utero from the actions of thyroid hormones, and in the sheep there appears to be a hierarchy of appearance of thyroid effects in various tissues as follows:

placenta>pancreas>pituitary>brain>bone>heart>lung>liver

Selected tissues are protected by rapid conversion of thyroxine to inactive analogues, by the delayed appearance of thyroid nuclear receptors and probably by programmed maturation of genetic expression and protein synthesis in response to thyroid hormone receptor activation.

Recent studies of thyroid hormone plasma production rates in the last third of gestation in the fetal sheep are summarized in Table I.[12] The predominant metabolites are T4 sulfate (T4S), reverse T3 sulfate (rT3S), and rT3. Reverse T3, rT3S, and T4S are biologically

Table 1. Iodothyronine Production in Fetal Sheep[a]	
Iodothyronine	Blood Production Rate (ug/kg/day)
T4	20-40
rT3	5
T3	2
T4S	10
rT3S	12
T3S	2
Bioinactive analogues rT3+T4S+rT3S	27
Bioactive analogues T3+T3S	4

From Polk et al, Am J Physiol 266:E892,1994

inactive so that only 10-15% of T4 in the fetus appears to be metabolized to active circulating thyroid hormone. Thus, thyroid metabolism in the third trimester sheep fetus is characterized by a relative deficiency of Type I, 5'iodothyronine monodeiodinase in peripheral tissues and by active sulfation of thyroid hormones and analogues, probably predominantly in liver. There is evidence that T3S can be desulfated in vivo in adult rats and may serve as a source of active T3 in the fetus, but T3S production also is limited.

It is known that brain maturation in the sheep fetus is thyroid hormone dependent from approximately 90 days (0.6) of gestation. The fraction of T4 metabolized to T3 in brain tissue during the last 0.4 of gestation is not known.

Thyroid Hormone Metabolism and Requirements in Premature Infants

Premature infants are born with an immature hypothalamic-pituitary-thyroid axis. As already discussed, neuroendocrine control of TSH secretion and thyroid gland TSH responsiveness mature during the latter half of human gestation (20-40 weeks), and there is a progressive increase during this time of serum TSH, TBG, T4 and free T4 concentrations with a plateau at 35-36 weeks.[13-17] Thyroxine production rate data during this period are not available. Premature infants are born with T4 and free T4 levels reflecting their gestation age and although there are increases in T4 and free T4 concentrations at the time of birth in response to a relatively obtunded TSH surge in the early minutes after birth, this transient (relative) hyperthyroxinemia subsides within 3-4 days and the hypothalamic-pituitary thyroid axis maturation continues to mature in the extrauterine environment. Over subsequent weeks there is a progressive increase in serum levels of T4, free T4, T3, free T3, and an increasing free T4/TSH ratio.

Fuse and colleagues from Tokyo characterized maturation of thyroid function

Table 2 Characteristics of the Longitudinal Study and Term Infants*		
Birthweight (Grams)	Gestation Age (Weeks)	No. Infants
769±112	25.0±1.6	12
1691±237	32.6±2.6	5
2240±158	35.3±0.7	9
3155±319 (Term)	39.0±1.1	131
*From Fuse Y et al, J Devel Physiol 14:17,1990		

parameters in 54 premature infants admitted to a neonatal nursery between April 1985 and March 1986.[18] Birthweights range from 588 to 2477 grams and gestation age from 23 to 39 weeks. A cohort of 131 term infants weighing 2516 to 3912 grams with gestation ages of 36-41 weeks also were studied. Twenty six of the infants were studied longitudinally with weekly testing for a period of 14 weeks. The characteristics of these infants are shown in Table 2. Figure 1 illustrates the pattern of maturation of serum free T4 and free T4/TSH ratios in the study infants. Thyroid control axis maturation resembled that in the in utero fetus during this 14 week period. Free T3 levels in these infants (not shown) increased progressively to the mid level of term infants by 14 weeks and maturation of the Free T3/TSH ratio resembled that of the free T4/TSH ratio.

Van Wassenaer and associates from the Academic Medical Center, Amsterdam treated 3 groups of premature infants ranging in age and weight from 25 to 29 weeks gestation and 650 to 1475 grams with varying doses of Na-l-thyroxine during the first 6 weeks of postnatal life.[19] Characteristics of the three groups of infants and dosage of thyroxine are summarized in Table 3. The three groups of infants were treated with 10 ug/kg/day (group 1), 8 ug/kg/day (group 2) or 6 ug/kg/day (group 3) of thyroxine, and plasma was drawn for thyroid hormone measurements at weekly intervals. A comparable group of control infants was included, but measurements were continued for only 3 weeks in these infants. The patterns of change in plasma free T4 concentrations in the three groups of infants are shown in Figure 2. The thyroxine therapy increased reverse T3 concentrations in proportion to the dosage but did not increase T3 concentrations relative to control infants. Plasma TSH levels were suppressed in all of the treatment groups and were maintained in the 4-7 mU/L range (for the 3 weeks of measurement) in control infants.

Table 3 Characteristics of the Three Premature Infant Treatment Groups				
	Gestation Age Mean/Range (Weeks)	Birthweight Mean/Range (Grams)	No Infants	Dose of T4 ug/kg/day
Group 1	27 26-29	1045 800-1300	13	10
Group 2	28 26-29	1170 880-1335	10	8
Group 3	28 25-29	1200 650-1475	10	6

Normal free T4 levels in premature infants were available for only 3 weeks in the Amsterdam infants so that the normal mean values developed by Fuse and colleagues in Tokyo are shown in Figure 2. The free T4 measurements were conducted in Amsterdam using a two step RIA method; Fuse et al utilized the Gamma Coat Clinical Assays kit (from Travenol Laboratories). The upper limits of the normal term infant range in the two studies were 35 and 65 pmol/L respectively. Thus a correction factor (0.53) was utilized to transpose the Fuse normative data to Figure 2. It is clear from Figure 2 that the normal T4 production rate is less than 6 ug/kg/day for premature infants of approximately 26-36 weeks gestation age in the extrauterine environment, based both on the extrapolated Fuse data and the suppressed serum TSH levels. An estimate of T4 production in untreated infants during this period of 2-4 ug/kg/day would seem reasonable.

Treatment Dosage of T4 in Congenital Hypothyroid Infants

There have been numerous studies of the efficacy of Na-l-thyroxine therapy of infants with congenital hypothyroidism (CH) detected in newborn screening programs during the past 2 decades. It is now clear that early adequate therapy will prevent or minimize the brain damage of CH as measured by IQ, school performance and neurological assessments.[20-23] The initial dosage of T4 in these studies has ranged from 6-15 ug/kg/day (usually 25-50 ug T4 daily in the average term infant). A recent study of Heyerdahl and colleagues is representative.[23] The mean serum T4 concentrations vs. T4 dose during the first 6 years in that study are summarized in Table 4. The initial average dose (8.5 ug/kg/day) produced a mean serum T4 levels of 193 nmol/L at 6 weeks of age. The target range for therapy (the upper half of the normal range) is 129 to 206 nmol/L (10 to 16 ug/dl). As noted in Table I, the dose in ug/kg/day to maintain the serum T4 level falls off progressively as the child grows. The average T4 replacement dose for a 70 kg adult with hypothyroidism is 1.5 ug/kg/day.

Table 4 Serum Thyroxine Concentrations Relative to T4 Dose in Children with Treated Congenital Hypothyroidism*		
Age	Mean Serum T4 (nmol/L)	Mean T4 Dose (ug/kg/day)
29 days**	40	8.5
6 wk	193	8.6
6 mo	166	5.0
1 yr	156	4.8
2 yr	160	4.4
6 yr	149	3.6
*From Heyerdahl et al, J Pediatrics 118:850, 1991 **Mean age at onset of therapy		

The indicated treatment dose in these children resulted in an average performance IQ measured at 6 years, of 94.4 ± 13.5 (mean and SD) for 46 children. The mean IQ of sibling pairs was 105 ± 16.4, a difference of 10 IQ points. In this study the 6 year IQ showed a correlation with mean serum T4 during the first year. Children with an average serum T4 < 129 nmol/L had a mean performance IQ of 82 at 6 years while those with a serum T4 level > 180 nmol/L had a mean 6 year IQ of 97.

In another study by Tillotson and colleagues from London, a total of 351 children born and screened between January 1982 and December 1984 were studied.[29] The T4 treatment dose ranged from 12 to 76 ug/day or about 2 to 8 ug/kg/day with a median approximating 5 ug/kg/day over the first year; average plasma T4 levels during the first year ranged from 79 to 261 nmol/L with a median approximating 170 nmol/L. Age at start of treatment or average plasma T4 level during the first year showed no correlation with 5 year IQ. There were positive correlations, however, between IQ and (non-manual vs. manual and unemployed) social class and between IQ and plasma T4 level (>40 nmol/L vs. <40 nmol/L) at the time of diagnosis. Glorieux, Dussault and VanVliet from Quebec reported significantly lower 12 year IQ values in children with pretreatment serum T4 levels < 26 nmol/L and retarded pretreatment bone age (n = 12) vs. children with higher T4 values and normal bone maturation at birth (n = 15, IQ values respectively 89 ± 17 vs. 104 ± 10).[29] In these children, the initial T4 treatment dose ranged from 6-8 ug/kg/day, and mean T4 values during the first year were 134 vs. 161 nmol/L, respectively in the lower vs. higher initial T4 groups.

These studies suggest that more severe (fetal) hypothyroidism is associated with an approximate 10 point IQ deficit relative to less severely affected infants in spite of early treatment. To exclude a dosage effect in their study Dubuis and coworkers from Dussault's group in Quebec studied a new cohort of CH infants and reported preliminary results from 12 infants, 7 with more severe and 5 with less severe CH at birth.[29] In this study the mean T4 dose at onset of therapy was 11.5 ug/kg/day (vs. 6-8 ug/kg/day in the

earlier study). The 18 month developmental quotient (Griffiths) values were 106 ± 10 and 113 ± 3 in the severe and less severe groups (vs. 98 ± 13 and 111 ± 5 in the earlier study). From a more extensive study of 45 CH infants, the same authors concluded that, with earlier treatment and a higher initial dose of levothyroxine (median 11.6 ug/kg/day), the early developmental outcome of infants with severe congenital hypothyroidism is now indistinguishable from that of infants with the moderate form of the disease used as controls.[27] These results and those of Heyerdahl and colleagues suggest that more intensive treatment during the first year of life can improve IQ in infants with more severe CH at birth.

Germak and Foley have reported results of longitudinal assessment of 34 CH infants treated with an initial dose of 10-14 ug/kg/day Na-l-thyroxine during the first year of life.[28] The average treatment dose over the first 6 months in infants with thyroid dysgenesis (excluding dyshormonogenesis) was 7 ug/kg/day; the average dose during the 7-12 month interval was 5.5 ug/kg/day. Average total T4 levels ranged from 130 to 206 nmol/L during the first 6 months and 130 to 167 nmol/L during the 7-12 month interval. Average free T4 levels ranged from 26-37 pmol/L during the first 6 months and 21-32 pmol/L during the second 6 months (normal newborn range 16-35 pmol/L). There were no developmental quotient data for these infants but the study documents the doses of Na-l-thyroxine necessary to maintain the serum T4 and free T4 levels in the upper half of the normal range in infants with congenital thyroid dysgenesis during the first year of life (11.5 ug/kg/day at birth, 7 ug/kg/day at 0-6 months and 5.5 ug/kg/day during the 7-12 month period).

From these several studies it is possible to develop a plot of thyroxine replacement dose/utilization rate versus age between 24 weeks gestation and 2 years of postnatal age. This plot is shown in Figure 3. The estimate for 24-40 weeks gestation, 2-4 ug/kg/day, was derived from Figure 2. Upper and lower ranges for this period probably are in the 1-6 ug/kg/day range. The remaining curve was developed from the data of Heyerdahl and colleagues, and Germak and Foley.[28,29] It is difficult to be precise in such estimates because data were derived from oral replacement therapy. Absorption may be incomplete and variable. Moreover, the thyroxine replacement doses in infants with CH were administered to maintain serum T4 and free T4 levels in the upper half of the normal range. Both incomplete absorption and the goal of maintaining high normal serum T4 concentrations would lead to overestimation of the endogenous T4 production/utilization rate in euthyroid infants. The dotted line in Figure 3 shows the estimated T4 utilization assuming a 70% average absorption rate.[29] There are no estimates of plasma production rates of T4 in human infants.

Iodine Requirements during Pregnancy, Infancy and Childhood

The daily intake of iodine recommended by the National Research Council (NRC-USA) in 1989 was 40 ug/day for young infants (0-6 months), 50 ug/day for older infants (6-12 months), 60-100 ug/day for children (1-10 years), and 150 ug/day for adolescents and adults.[30] These values, converted to averages in ug/kg/day approximate 7,5.5,4 and 2

ug/kg/day respectively. These amounts are proposed to allow normal T4 production without stressing the thyroid iodide trapping mechanism or raising TSH levels.

The appropriateness of these recommendations has been recently further discussed and partly questioned.[31] The figure of 150 ug iodine/day for adolescents an adults is justified by the fact that it corresponds to the daily urinary excretion of iodine and to the iodine content of food in non endemic areas.[32] It also provides the iodine intake necessary to maintain the plasma iodide level above the critical limit of 0.10 ug/dl, which is the average level likely associated with the onset of goiter.[33] Moreover, this level of iodine intake is required to maintain the iodine stores of the thyroid above the critical threshold of 10 mg, below which an insufficient level of iodination of thyroglobulin initiates disorders in thyroid hormone synthesis.[34]

The iodine requirement during pregnancy is increased to provide for the needs of the fetus and to compensate for the increased loss of iodine in the urine due to increased renal clearance of iodide during pregnancy.[35] These requirements have derived from studies of thyroid function during pregnancy and in the neonate under conditions of moderate iodine deficiency; an example is Belgium where the iodine intake is estimated at 50-70 ug/day.[36] In such an environment, thyroid function during pregnancy is characterized by a progressive decrease of the serum concentrations of thyroid hormones and an increase in serum TSH and thyroglobulin. Thyroid volume progressively increases and is above the upper limit of normal in 10% of the women by the end of pregnancy. Serum TSH and thyroglobulin are still higher in the neonates than in the mothers.[37] These abnormalities are prevented only when the mother receives a daily supplementation of 165 ug iodide/day during pregnancy (100 ug/kg and 100 ug thyroxine).[36] These data indicate that the iodine intake required to prevent the onset of subclinical hypothyroidism of mother and fetus during pregnancy, and thus to prevent the risk of brain damage of the fetus, is in the range of 200-250 ug/day.

The US recommendation of 40 ug iodine per day in infants aged 0 to 6 months (or 8 ug/kg/day, 7 ug/100 Kal, 5 ug/dL milk) derives probably for the observation that, up to the late sixties, the iodine content of breast milk approximated 5 ug/dL and from the concept that nutrition of the breast fed infant growing at a satisfactory rate has been the standard against which nutrition requirements have been set.[38,40] However, more recent data indicate that the iodine content of breast milk varies markedly as a function of the iodine intake of the population. For example, it ranges from 2 to 33 ug/dL in Europe and from 3 to 49 ug/dL in the United States.[39,40] It is as low as 1.2 ug/dL in conditions of severe iodine deficiency.[39,40] An average breast milk intake of 700 ml daily would give an intake of iodine of about 56 ug/day in Europe and 112 ug/day in the United States. The upper United States value (49 ug/dL) would provide 343 ug/day or 68 ug/kg/day for a 5 kg infant. Iodine balance in the young infant, which is required to accommodate the increasing iodine stores of the thyroid, is achieved only when the iodine intake is at least 15 ug/kg/day in full term and 30 ug/kg/day in preterm infants.[41] This corresponds approximately to an iodine intake of 90 ug/day, a value 2 fold higher than the US recommendations. Based on these considerations, the present recommendation by WHO

is an iodine intake of 90 ug/day from birth onwards.[42] To reach this objective, and based on an intake of milk of about 150 ml/kg/day, the iodine content of formula milk should be increased from 5 ug/dL to 10 ug/dL for full term and 20 ug/dL for preterm infants. Considering a urine volume of about 4-6 dL per day from 0-3 years, the urinary concentration of iodine indicating iodine repletion should be in the range of 15-22 ug/dL in infants aged 0-36 months. Such values have been observed in iodine replete infants in Europe[43] in Canada[43] and in the United States.[44] In conditions of moderate iodine deficiency as seen in Belgium, the average urinary iodine concentration is only 5-10 ug/dL in this age group and reaches a stable normal value of 18-22 ug/L only after several months of daily supplementation by a physiological dose of 90 ug/kg/day. Figure 4.

When the urinary iodine concentration in neonates and young infants is below a threshold of 5-6 ug/dL corresponding to an intake of 25-35 ug/day, there is a sudden increase in the prevalence of neonatal serum TSH values in excess of 50 mU/L indicating subclinical hypothyroidism and eventually complicated by transient neonatal hypothyroidism.[45] When the urinary iodine concentration is in the range of 1-2 ug/dL, as observed in severe endemic goiter regions, up to 10% of the neonates have overt severe hypothyroidism with serum TSH levels above 100 mU/mL and serum T4 values below 3 ug/dL (39 nmol/L).[45] Untreated, these infants progress to the full picture of myxedematous endemic cretinism.[46]

Thus, the iodine requirement of the young infant approximates 15 ug/kg/day (30 ug/kg/day in preterms); hyperthyrotropinemia, indicating subclinical hypothyroidism with the risk of brain damage, occurs when the iodine intake is about one third of this value, and dramatic neonatal hypothyroidism resulting in endemic cretinism occurs when the intake is about one tenth of this value.

The daily iodine need on a body weight basis decreases progressively with age. A study by Tovar and colleagues[47] correlating 24 hour thyroid radioiodine uptake and urinary iodide excretion in 9-13 year old schoolchildren in rural Mexico suggested that an iodine intake in excess of 60 ug/day is associated with a 24 hour thyroidal radioiodine uptake below 30%. Lower excretion values are associated with higher uptake values. This would approximate 3 ug/kg/day in an average size 10 year old so that 60-100 ug daily intake in a 1-10 year old child would seem appropriate.

Figure 5 summarizes iodine needs as recommended by the NRC and by the authors respectively.

Iodine excess also can be harmful to the thyroid of infants by inhibiting the process of synthesis and release of thyroid hormones (Wolff - Chaikoff effect).[48] The threshold upper limit of iodine intake to inhibit thyroid function is not easy to define because it is conditioned by the level of iodine intake before exposure to iodine excess. Indeed, long standing moderate iodine deficiency is accompanied by an accelerated trapping of iodide and by a decrease in the iodine stores within the thyroid.[49] In these conditions, the critical ratio between iodide and total iodine within the thyroid, which is the starting point

of the Wolff-Chaikoff effect, is more easily reached in conditions of iodine depletion than in normal conditions.[48] In addition, the neonatal thyroid is particularly sensitive to the Wolff-Chaikoff effect because the immature thyroid is unable to decrease iodide clearance to compensate for increased iodine intake.[49]

It is for these reasons that transient neonatal hypothyroidism or transient hyperTSHemia following iodine overload of the mother, especially after the use of povidone iodine, has been reported more frequently in European countries such as in Belgium, France, and Germany which have prevailing moderate iodine deficiency.[50-53]

In a study in Belgium, iodine overload of mothers (cutaneous povidone iodine) increased the breast milk iodine concentration and increased newborn iodine excretion of term infants (mean weight about 3 kg). Mean milk iodine concentrations of 18 ug/dL and 128 ug/dL were associated with average infant urinary iodine excretion levels of 28 ug/dL and 184 ug/dL, respectively.[50] Estimated average iodine intakes would be 112 and 736 ug daily, or 37 and 245 ug/kg/day, respectively. The lower dose significantly increased the peak TSH response to exogenous TRH but did not increase the (secretory) area under the TSH response curve. The larger dose increased both the peak response and secretory area as well as the baseline TSH concentration. Serum thyroxine concentrations were not altered, however. Thus, these infants had a mild and transient, compensated hypothyroid state. Non contaminated mothers secreted breast milk containing 9.5 ug of iodine/dL, and the mean urinary iodine concentration of their infants was 14.4 ug/dL. These data indicate that modest iodine overloading of term infants in the neonatal period in an area of relative dietary iodine deficiency (Belgium) also can impair thyroid hormone secretion.

Similarly, studies in France indicated that premature infants exposed to cutaneous povidine iodine or fluorescinated alcohol-iodine solutions, and excreting iodine in urine in excess of 100 ug daily, manifested decreased thyroxine and increased TSH concentrations in serum.[52] The extent of these changes was more marked in infants less than 34 weeks gestation than in 35-37 week premastures; full term infants were not affected. These studies suggest that in Europe the upper limit of iodine intake which predisposes to blockage of thyroid secretion in premature infants (about 200 ug daily) is 2 to 3 times the average intake from breast milk, and about equivalent to the upper range of intake.

Similar studies have not been conducted in the United States where transient hypothyroidism is rarely seen, perhaps because the iodine intake is much higher. For example, urinary concentrations in neonates of 50 ug/dL and above which can correspond to a Wolff-Chaikoff effect in Europe are frequently seen in healthy neonates in North America.[43, 44]

The average iodine intake of infants in the United States in 1978, including infants fed whole cow's milk, was estimated by the "market basket" approach.[54] The mean intake by infants was 576 ug/day (standard deviation 196) and that by toddlers was 728 ug/day (SD 315). The corresponding value for adults was 952 ug/day (SD 589). The upper range for infants (968 ug/day) would provide a daily intake of 138 ug/kg for a 7 kg infant,

and the upper range for toddlers (1358 ug/day), would provide a daily intake of 90 ug/kg for a 15 kg toddler.

Table 5 summarizes the recommended dietary intake of iodine for age and approximate level of intake which appear not to impair thyroid function in the European studies of Delange in infants, in the loading studies of adults in the United States, or during ingestion of the highest estimates of dietary intake (just reviewed) in the United States. With the exception of the values for premature infants, these "probably safe" limits are 15 to more than 20 times the recommended intakes. These data refer to all sources of iodine intake. The average iodine content of infant formulas approximates 5 ug/dL or 50 ug/L. The upper limit probably should be one that provides a daily iodine intake of no more than 100 ug/kg. Given this limit and assuming the total intake to be formula, with a daily intake of 150 ml/kg (100 Kcal/kg), the upper limit of the iodine content of formula would be about 65 ug/dL. The current suggested upperlimit of iodine in infant formulas of 75 ug/100 Kcal (50 ug/dL) would seem reasonable.

Table 5
Recommended Dietary Intakes of Iodine and Probable Safe Upper Limits +

Age	Dietary Iodine Intake (ug/kg/day)	
	Recommended	Upper Limit*
Premature Infants	30	100
Infants 0-6 Months	15	150
Infants 0-12 Months	7	140
Children 1-10 Years	3	50
Adolescents/Adults	2	30

*Probably Safe
+ See Text for Details

Summary and Conclusions

The low free T4 levels and low production rate of T4 in the premature infant represent a state of relative hypothalamic immaturity since the responses to TRH and TSH are intact, excluding pituitary and thyroid deficiency. There is no evidence that this transient hypothalamic immaturity leads to marked IQ damage in the healthy premature infant. However, a 5-10 point IQ deficit cannot be excluded.

The studies of Tillotson and coworkers, Heyerdahl et al, and the Quebec investigators suggest that supraphysiological levels of thyroxine maintained during the first year of life can increase IQ value by some 10 points in infants presenting at birth with evidence of intrauterine hypothyroidism (delayed bone maturation and low serum T4 concentrations, < 40 nmol/L).[28-30] This concept is illustrated in Figure 6 and indicates that the period of

thyroxine dependency of brain maturation includes the third trimester of gestation. Fetal thyroid hormone deficiency during this period appears to account for approximately 10 points of IQ. Severe thyroid hormone deficiency during the first 6 months of life leads to an average loss of some 30 points of IQ. The extent to which the entire curve of IQ loss versus age is shifted to the left and downward by severe fetal hypothyroidism and the time period of intrauterine brain vulnerability are not yet clear.

The data from Figures 3 and 5 indicate that the threshold iodine need of 6 to 14 ug/kg/day during the first 2 years of life compares to a thyroxine utilization rate of 3 to 7 ug/kg/day. Since thyroxine is 64% iodine by weight, iodine need exceeds thyroxine iodine turnover in the infant with normal, nonstressed thyroid function by 3-4 fold. This is due to thyroid follicular cell immaturity. Thyroid gland function in the late gestation fetus and premature infant is characterized by low concentrations of thyroglobulin per mg thyroid tissue, relatively high concentrations of thyroidal inorganic iodine, low organic iodine stores, and high rates of thyroglobulin secretion.[15] Thus, organification appears to be relatively inefficient making the premature infant more susceptible to hypothyroidism induced either by iodine deficiency or excess. Delange et al. have recommended an intake of iodine of 30 ug/kg/day in premature infants.[16] The term infant in areas of marginal iodine intake also is at risk for transient hypothyroidism.[24]

The risk of iodine overload producing hypothyroidism with iodine supplementation is significant in the premature infant where the recommended and upper limits of iodine intake differ by only 3-4 fold.(Table 5) In full term infants and children the recommended and upper limits differ by 10-20 fold and usually encountered or supplemented dietary intakes would provide little risk of overload.

As shown in Figure 2 thyroid hormone utilization/production in the premature infant appears to be relatively low; serum FT4 levels are raised considerably above control levels and serum TSH values are suppressed by a T4 dose of 6 ug/kg/day. A T4 production rate in the "normal" premature infant of 3-4 ug/kg/day was estimated (Figure 3). This low thyroxine production/utilization in the premature infant is consistent with the observation that most infants with CH and low cord serum T4 levels (averaging 30 nmol/L in the athyroid fetus) appear normal at birth and with early treatment manifest normal IQ values at 6 and 10 years. The reason(s) for the marked increase in T4 and T3 production/utilization in the neonatal period in the term infant is not clear. Increased endogenous thermogenesis and increased pulmonary, hepatic and carotid perfusion presumably contribute. It is also unclear why supraphysiological doses of thyroxine during the first year improve IQ in the infant with severe CH. It would appear that mild hyperthyroidism relatively accelerates the processes of CNS maturation during the extrauterine period of thyroxine dependency.

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Figure 5

Suggested threshold and National Research Council (NRC, USA) recommended iodine intake during infancy and childhood. The suggested threshold needs were developed by the authors as described in text.

Figure 6

Intelligence quotient (IQ) plotted vs. age for infants with congenital hypothyroidism of severe or moderate degree with and without treatment. With low dose or higher dose thyroxine replacement, infants with moderate disease usually manifest normal IQ values at 6-8 years of age. Infants with severe disease treated with low dose thyroxine (6-8 ug/kg/day) manifest 6-8 year IQ values averaging 10 points below values in infants replaced with 10-15 ug/kg/day thyroxine daily at birth. See text for details.

mjm brain.doc

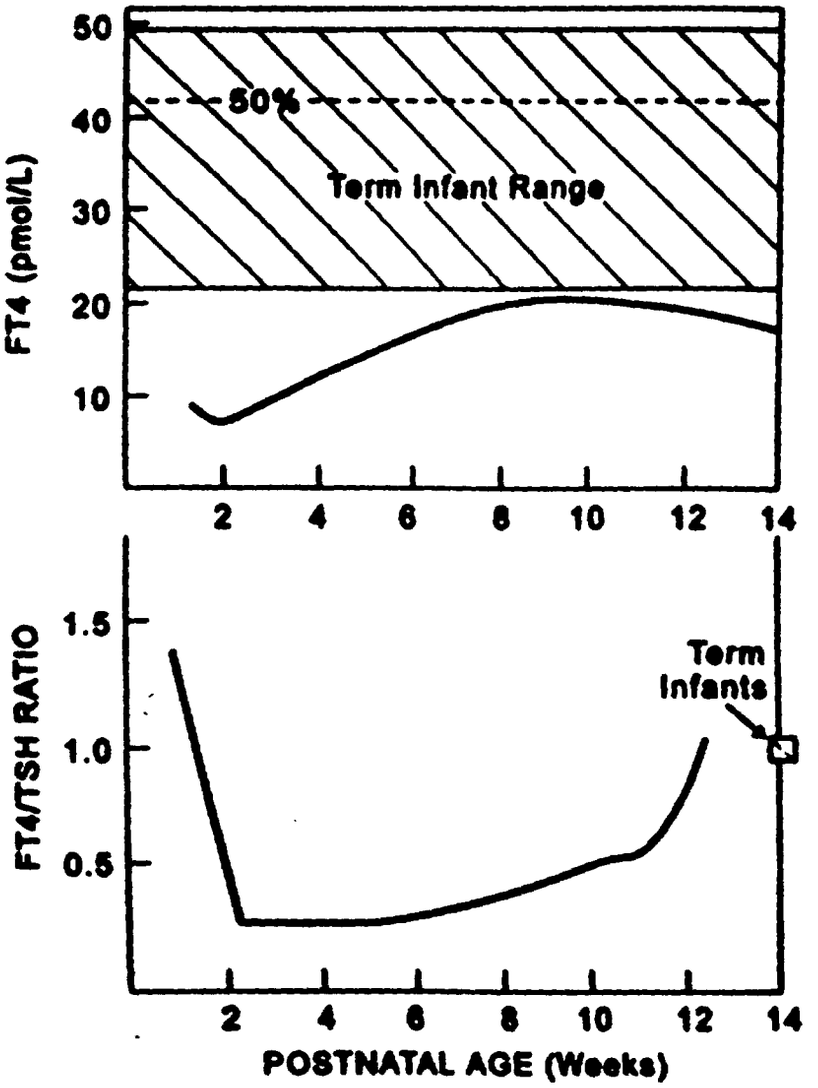

50
40
30
20
10
50%
Term Infant Range
FT4 (pmol/L)
2
4
6
8
10
12
14
1.5
1.0
0.5
FT4/TSH RATIO
Term
Infants
POSTNATAL AGE (Weeks)

Fig 1

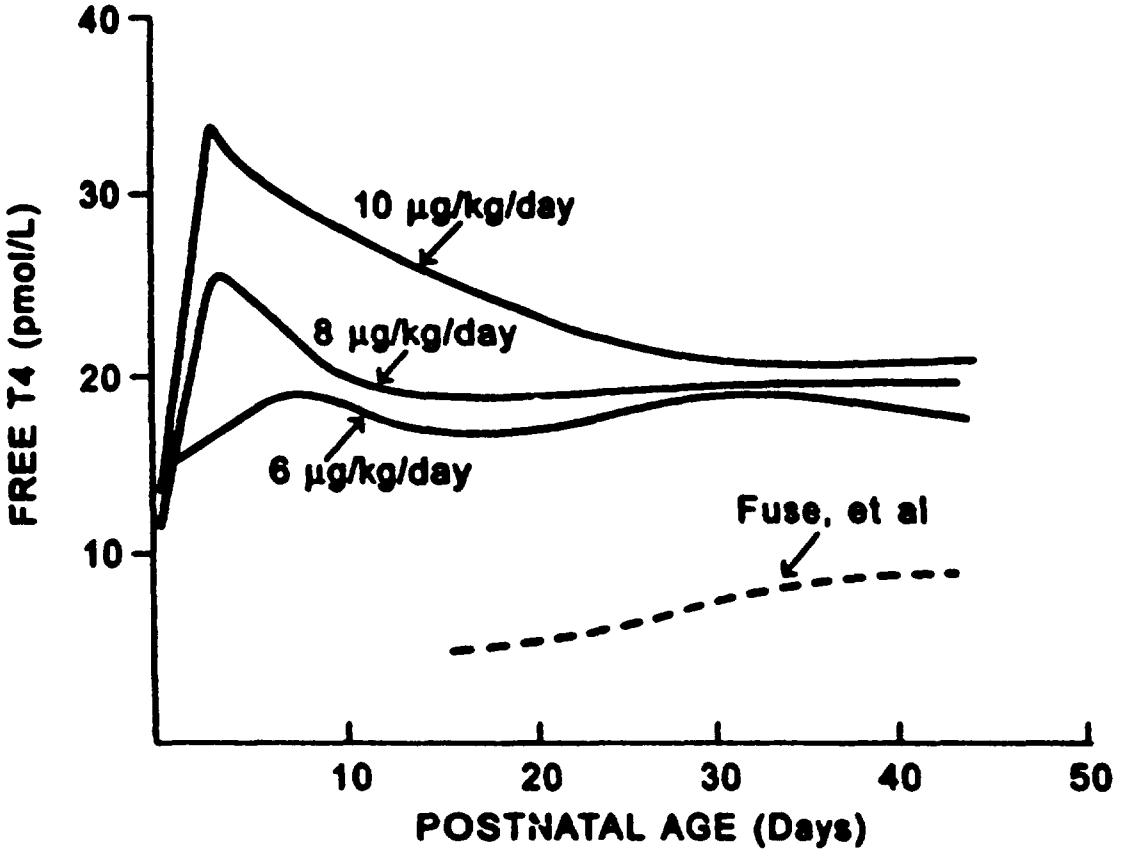

40
30
20
10
FREE T4 (pmol/L)
10 µg/kg/day
8 µg/kg/day
6 µg/kg/day
Fuse, et al
10
20
30
40
50
POSTNATAL AGE (Days)

Fig 2

Attachment 4

INFORMATION ON PERCHLORATE HEALTH EFFECTS COMPILED FOR THE PERCHLORATE STUDY GROUP

AS DEFINED BY EXPERT WORK GROUPS

An RfD or RfC is

...an estimate (with uncertainty spanning perhaps an **order of magnitude**) of

a **daily** (for RfD) **or continuous** (for RfC) exposure to the human population (including **sensitive** subgroups)

that is **likely to be without** an appreciable risk of deleterious effects during a lifetime.

The estimation of these subthreshold doses involves several judgments

such as...

the **choice of** the most appropriate No Observed Adverse Effect Level (**NOAEL**) of the critical effect, usually from animal data, and

the **choice of** the appropriate **uncertainty factors** based on a review of the entire database.

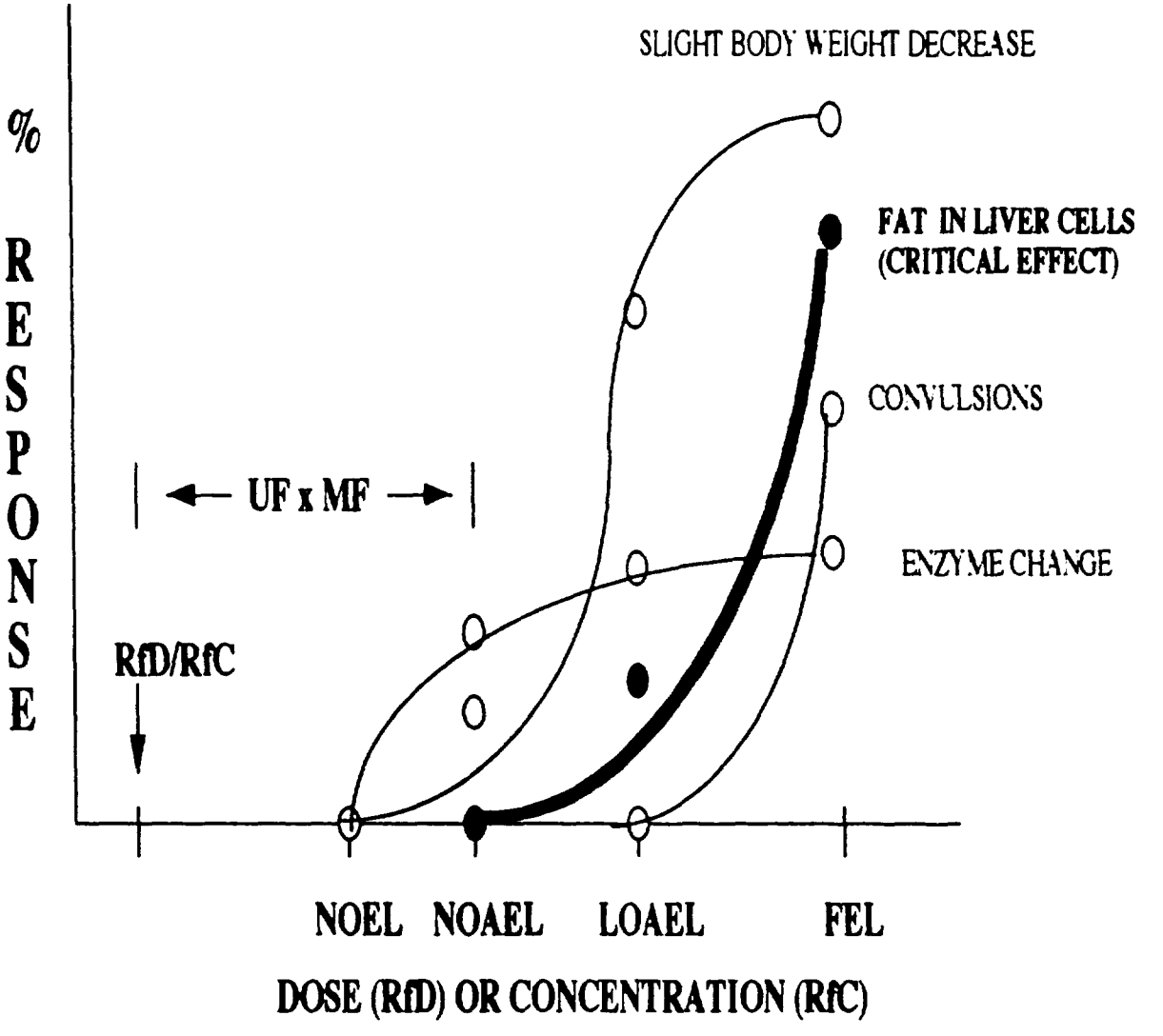


FIGURE 1. The judgement of the critical effect and its NOAEL along with the appropriate uncertainty and modifying factors leads to the estimation of the RfD or RfC. As the dose or concentration exceeds the RfD or RfC, the probability of adverse effects increases.

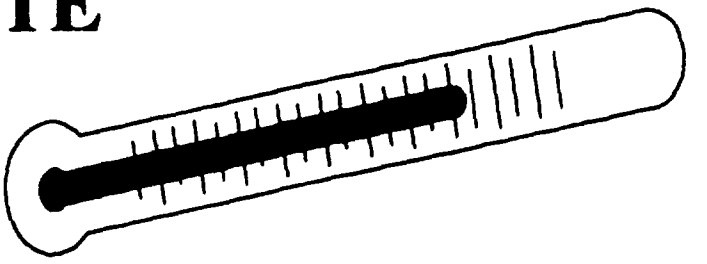
UNCERTAINTY FACTORS

FACTOR*		EXTRAPOLATION
H	10	AVERAGE HUMAN TO SENSITIVE HUMAN
A	10 or 3**	ANIMAL TO HUMAN
S	≤10	SHORT TERM TO LONG TERM EXPOSURE
L	≤10	LOAEL TO NOAEL
D	≤10	MINIMUM TO COMPLETE DATA BASE

*These factors are as used by the U.S. EPA. Other health organizations use similar factors. In EPA, The maximum UF for any given data base is 10,000. Data bases weaker than this are judged too uncertain to estimate RfD/RfCs.

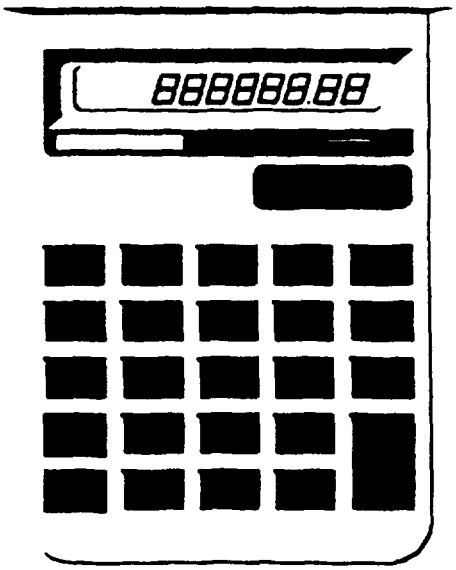
**For RfCs the default UF for area "A," animal to human, is 3.

HOW ACCURATE ARE THESE ESTIMATES?



ADIs, MRLs, RfDs, RfCs or TIs are considered to be accurate estimates of doses **below a toxicity threshold,** because they are based on a review of all toxicity data and individual uncertainty factors are considered to be somewhat protective. The use of several UFs can yield conservative estimates.

....If you play golf, accurate drives are generally those that land in the fairway, although they may be scattered over a wide area.



HOW PRECISE ARE THESE ESTIMATES?

Not very! Each uncertainty factor varies with ranges up to about 10-fold. Several factors are generally multiplied to estimate a subthreshold dose and some factors overlap, thereby increasing variability and decreasing precision.

.....If you play golf, precise drives are those that consistently land in one area of the fairway (or the creek).

Major assumptions, strengths & limitations of the RfD/RfC

Major assumptions are...

a population threshold exists,

these estimates represents subthreshold doses, and

preventing the critical effect protects against all effects.

Major strengths are...

all data are reviewed in the choice of the critical effect and its NOAEL

uncertainties in the entire data base can be addressed in the RfD/RfC thru factors based on best judgement

Major limitations are...

NOAEL of the critical effect ignores much of the data and often does not distinguish amongst better quality studies,

uncertainty factors are imprecise, and

risks above these estimates of subthreshold doses are not estimated.

Issues for Proposed Perchlorate RfD

- An abundance of data exists on perchlorate's effects on Graves' patients, but data are more limited on chronic exposures in normal humans and animals at concentrations likely found in environmental exposures. Although the thyroid appears to be the principal target organ, no other organ systems have been well-studied in animals. Other effects seen in Grave's patients are hematological at doses 100-fold higher than those needed to affect iodine concentrations in the thyroid. Therefore, does the database support the development of an RfD for perchlorate?

- Are immediate effects on the thyroid (i.e., inhibition of iodine uptake and discharge of iodine from thyroid) the appropriate critical effect for the RfD?

- Which of the available data best reflect expected effects in normal or sensitive humans?

Questions Related to the Uncertainty Factor:

- Do rats respond faster and to a greater degree than humans to the effects of perchlorate because the rat thyroid lacks an iodide binding protein, resulting in greater effort to maintain constant blood levels of thyroid hormones? Does this response mean that rats are more sensitive than humans to effects of perchlorate? How does this affect the uncertainty factor for interspecies extrapolation?

- Long-term animal studies at very high doses show the types of morphological changes in the thyroid typical of a prolonged exposure to TSH that is seen after chronic interference with the thyroid-pituitary axis. How might this affect the uncertainty factor for subchronic-to-chronic exposures?

- Can Graves' patients be considered sensitive when compared to normal humans to both effects on the thyroid and hematological effects?

- Thyroid hormones play a critical role in the neurological development of infants up to two years of age. However, there are no studies which evaluated the role of perchlorate on the neurological development of neonates. Are neonates considered a sensitive subpopulation for perchlorate? Will the uncertainty factor of three protect neonates from the effects of perchlorate?

- The addition of Caldwell (1996) strengthens the database; how does this affect the use of a database uncertainty factor?

Noncancer Oral Level 3

Chemical Perchlorate

CAS Number 7778-74-7

Organization Toxicology Excellence for Risk Assessment

Risk Value and Name 1E-2 mg/kg/day Reference Dose (RfD)

Year: 1997

Determination of Critical Effect

Until the mid 1960's perchlorate was used to treat hyperthyroidism caused by Graves' disease. Therefore, perchlorate has been extensively studied in Graves' disease patients and to a lesser extent in normal humans. In addition, both short- and long-term studies in various rodent species have been conducted. The data in both humans and animals indicate that perchlorate exerts its effects by competitively inhibiting uptake of iodine into the thyroid thereby inhibiting the production of iodine-containing thyroid hormones. The short-term consequence of this action is a response by the pituitary gland to produce TSH which in turn stimulates diffuse cell division and growth of the thyroid gland. Effects related to disturbance of the thyroid-pituitary axis have been seen in studies in humans, both Graves' patients and normal humans, and in both short-term and long-term studies in animals. Thus, disturbance of the function of the thyroid-pituitary axis appears to be the critical effect from exposure perchlorate.

The human studies demonstrate that Graves' disease patients treated with doses in the range of 6 to 14 mg/kg/day occasionally developed fatal aplastic anemia (Hobson, 1961; Johnson and Moore, 1961; Fawcett and Clark, 1961; Krevans et al., 1962; and Gjemdal, 1963). However, this response is likely to be the result of patients with an improperly functioning immune system suffering an immune mediated hypersensitivity reaction to perchlorate. In a single Graves' disease patient, a dose level of 3 mg/kg/day controlled hyperthyroidism with no side effects after 22 years of treatment (Connell, 1981). When the underlying mechanism of toxicity is examined (i.e., prevention of iodine uptake by the thyroid), a dose of 1.4 mg/kg/day in Graves' patients caused complete release of iodine by the thyroid while lower doses caused only a partial release (Stanbury and Wyngaarden, 1952). This dose represents the lowest LOAEL observed in the human database; no human studies which identify a dose that has no effect at all on thyroid function were found.

The critical study, Stanbury and Wyngaarden (1952), evaluated perchlorate in patients with Graves' disease and found that perchlorate caused the discharge of iodine accumulated in the thyroid and blocked the uptake of iodine into the thyroid. Within 30 minutes of administration, a single dose of 100 mg potassium perchlorate (1.4 mg/kg/day) caused the nearly complete release (~80%) of I-131 from the thyroids of 8 Graves' disease patients previously treated with tracer amounts of I-131 and 1-methyl-2-mercaptoimidazole (MMIA). MMIA is an antithyroid agent that inhibits incorporation of iodide into thyroid hormone molecules. Pretreatment with MMIA ensured that any I-131 accumulated in the thyroid was not used to produce thyroid hormone. A single dose of 10 mg perchlorate (0.14 mg/kg/day) appeared to cause about a 50% release of accumulated iodine and the authors reported that perchlorate doses as low as 3 mg (0.04 mg/kg/day) caused detectable, but incomplete, release of iodine from the thyroid (data for doses less than 10 mg were not presented). In addition, Stanbury and

Wyngaarden (1952) reported that the uptake of tracer levels of I-131 into the thyroid glands of ients with Graves' disease was markedly inhibited for as long as 6 hours when 100 mg of assium perchlorate was given orally 1 hour prior to administration of the tracer. Inhibition of iodine occurred both in two patients treated with MMIA and three patients without MMIA treatment.. The authors state that no toxic effects were encountered in any of these patients who were given no more than three doses for a total of not more than 600 mg potassium perchlorate. . This study identifies a definitive LOAEL of 1.4 mg/kg/day for complete release of iodine from the thyroid. Since it is not clear what degree of iodine release constitutes an adverse effect, a NOAEL was not designated for this study.

Most animal studies were conducted at doses that were too high to identify the threshold for perchlorate's effect on the thyroid-pituitary axis. However, two animal studies identified NOAELs. A four-day study identified a NOAEL of 1.5 mg/kg/day (Mannisto et al., 1979) and a 14-day study identified a NOAEL of 0.12 mg/kg/day (this dose was a LOAEL in females) based on decreased thyroid hormone levels and increased TSH levels (Caldwell et al., 1996). A third study suggested a NOAEL of 0.25 mg/kg/day for increased secretion of iodine from the thyroid. However, this study was poorly reported and lacked critical information necessary to be adequate for risk assessment purposes (Shigan, 1963). A defect of the animal studies is that none of the studies adequately examined organs or tissues other than the thyroid.

Quantitative Estimate

The RfD was calculated based on a LOAEL of 1.4 mg/kg/day (Stanbury and Wyngaarden, 52) and a total uncertainty factor of 100. Because no human studies have identified a definitive NOAEL; the RfD is based on the lowest human LOAEL identified. This study is supported by short-term animal studies which find NOAELs in the range of 0.1-1.5 mg/kg/day. Uncertainty factors include 3 to protect sensitive subpopulations, 3 to account for extrapolation from short-term studies, 3 to account for database deficiencies and 3 for the use of a minimal LOAEL. Confidence in the RfD is medium-to-low because of the general lack of information about the effects of chronic exposure at low doses.

Peer Review

This RfD was reviewed at a *TERA* peer review meeting on March 7, 1997.

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Proposed Perchlorate Reference Dose (RfD)

Prepared for:

The Perchlorate Study Group (PSG)

Prepared by:

Toxicology Excellence for Risk Assessment

Peer Review Draft February 1996

Reference Dose (RfD) for Perchlorate

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Executive Summary

A reference dose (RfD) of 0.01 mg/kg/day is proposed for perchlorate. This RfD is based on a critical study in sensitive humans which identifies a lowest observed adverse effect level (LOAEL) of 1.4 mg/kg/day based on early manifestations of thyroid-pituitary disturbance. No human studies have identified a definitive no observed adverse effect level (NOAEL); the RfD is based on the lowest human LOAEL identified. This study is supported by short-term animal studies which find NOAELs in the range of 0.1-1.5 mg/kg/day. Recommended uncertainty factors include 3 to protect sensitive subpopulations, 3 to account for extrapolation from short-term studies, 3 to account for database deficiencies and 3 for the use of a minimal LOAEL. Confidence in the RfD is medium-to-low because of the general lack of information about the effects of chronic exposure at low doses.

Until the mid 1960's perchlorate was used to treat hyperthyroidism caused by Graves' disease. Therefore, perchlorate has been extensively studied in Graves' disease patients and to a lesser extent in normal humans. In addition, both short- and long-term studies in various rodent species have been conducted. The data in both humans and animals indicate that perchlorate exerts its effects by competitively inhibiting uptake of iodide into the thyroid thereby inhibiting the production of iodide-containing thyroid hormones. The short-term consequence of this action is a response by the pituitary gland to produce TSH which in turn stimulates diffuse cell division and growth of the thyroid gland. Effects related to disturbance of the thyroid-pituitary axis have been seen in studies in humans, both Graves' patients and normal humans, and in both short-term and long-term studies in animals. Thus, disturbance of the function of the thyroid-pituitary axis appears to be the critical effect from exposure to perchlorate.

The human studies demonstrate that Graves' disease patients treated with doses in the range of 6 to 14 mg/kg/day occasionally developed fatal aplastic anemia. However, this response is likely to be the result of patients with an improperly functioning immune system suffering an immune mediated hypersensitivity reaction to perchlorate. In a single Graves' disease patient, a dose level of 3 mg/kg/day controlled hyperthyroidism with no side effects after 22 years of treatment. Thus, this dose, while having a beneficial effect in Graves' patients, might be a LOAEL in normal humans with a lifetime of perchlorate exposure. When the underlying mechanism of toxicity is examined (i.e., prevention of iodide uptake by the thyroid), a dose of 1.4 mg/kg/day (LOAEL) in Graves patients caused complete release of iodide by the thyroid while lower doses caused only a partial release. No human studies which identify a dose that has no effect at all on thyroid function were found.

Most animal studies were conducted at doses that were too high to identify the threshold for perchlorate's effect on the thyroid-pituitary axis. However, two animal studies identified NOAELs. A four day study identified a NOAEL of 1.5 mg/kg/day and a 14-day study identified a NOAEL of 0.12 mg/kg/day (this dose was a LOAEL in females) based on decreased thyroid hormone levels and increased TSH levels. A third study suggested a NOAEL of 0.25 for increased secretion of iodide from the thyroid. However, this study was poorly reported and inadequate for risk assessment purposes. A defect of the animal studies is that few of the studies examined any organs or tissues other than the thyroid.

1. Introduction

Perchlorate compounds have been widely used as solid rocket propellants and ignitable sources in munitions and fireworks. Perchlorates are also a laboratory waste by-product of perchloric acid. Because perchlorate use was required in the performance of Department of Defense and National Aeronautic and Space Administration contract, government and contractor facilities are potential locations requiring extensive perchlorate remediation. These compounds have been found as contaminants in soils and groundwater. In addition, until recently, perchlorate salts, particularly potassium perchlorate, have been used therapeutically to treat hyperthyroidism resulting from Graves' disease. Perchlorate, ClO_4^-, is an anion which forms salts with most cations. These salts dissociate completely when dissolved in water or aqueous tissues.

This paper will discuss the human and animal toxicity data for perchlorates and calculate an oral reference dose (RfD) for the non-cancer health endpoints following the U.S. Environmental Protection Agency (U.S. EPA) methods. Several important issues related to perchlorates' potential for causing adverse health effects in humans will be discussed to better characterize the health risk

1.1 Existing Provisional Reference Dose (RfD)

RfDs for perchlorate-containing compounds, including potassium perchlorate (CAS# 7778-74-7), ammonium perchlorate (CAS# 7790-98-9), lithium perchlorate (CAS# 7791-03-9), sodium perchlorate (CAS# 7601-89-0) or perchloric acid (CAS# 7601-90-3) are not available on U.S. EPA's Integrated Risk Information System (IRIS) or Health Effects Assessment Summary Tables (HEAST). In late 1992, U.S. EPA's Superfund Health Risk Technical Support Center in the National Center for Environmental Assessment (NCEA) assessed the toxicity of potassium perchlorate and developed a provisional RfD for the perchlorate compounds. This provisional value has been used as the basis for developing clean-up levels by U.S. EPA Regional Superfund personnel. In addition, U.S. EPA Region III has placed this provisional value on its Risk-Based Concentration Tables, which are a widely-used risk assessment reference for many state agencies.

The provisional RfD is based on an acute study by Stanbury and Wyngaarden (1952) in which single doses of potassium perchlorate caused the release of iodide from the thyroids of patients with Graves' disease. The NOAEL was determined to be 0.14 mg/kg/day because iodide release was incomplete at

this dose. The 1000-fold uncertainty factor included a factor of 10 for the use of a less-than-chronic study, 10 to protect sensitive subpopulations, and 10 to account for database deficiencies. The resulting provisional RfD was 0.0001 mg/kg/day.

1.2 Purpose of this Document

In 1995, the Perchlorate Study Group (a consortium of companies that use and/or manufacture perchlorates) submitted a revised assessment of the perchlorate RfD to U.S. EPA-NCEA for review. At that time, several issues regarding the association of perchlorate treatment with fatal hematological disorders and the deficiencies in the overall database were identified and remained unresolved. The purpose of this document is to develop an RfD for perchlorate based on a comprehensive discussion of its likely critical effect and uncertainty factors that incorporates the latest information on interhuman variability, interspecies extrapolation, extrapolation across durations, and strengths and limitations of the overall database. These issues are discussed below.

1.3 The Method Used

The RfD method of U.S. EPA was used to evaluate and quantitate the non-cancer toxicity of perchlorate. The determination of RfDs lies squarely in the area of hazard identification and dose response assessment as defined by the National Academy of Sciences (NAS, 1983) report on risk assessment in the federal government. U.S. EPA defines the reference dose as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. (Barnes and Dourson, 1988; Dourson, 1994).

For health effects that are not cancer, the U.S. EPA and others first identify the critical effect(s), which is the first adverse effect(s) or its known precursor that occurs in the dose scale. Human toxicity data adequate for use in the estimation of RfDs are seldom available, but if so, they are preferred in the selection of this critical effect. The use of human data has the advantage of avoiding the uncertainties inherent in interspecies extrapolation.

After the critical effect(s) has been identified, U.S. EPA generally selects an experimental dose rate from a study that represents the highest level tested at which the critical effect was not demonstrated. This level (i.e., the NOAEL) is

the key datum gleaned from the toxicologist's review of the chemical's entire database and is the first component in the estimation of an RfD. If a NOAEL is not available, the use of a LOAEL is recommended. Alternatively, a benchmark dose (BMD) may be used in this part of the assessment. A BMD is a statistical lower confidence limit on the dose that produces a predetermined level of change in adverse response compared with the response in untreated animals (called the benchmark response or BMR). Advantages and disadvantages of NOAELs and BMDs are described elsewhere (U.S. EPA, 1995).

Presented with data from several animal studies, U.S. EPA first seeks to identify the animal model that is most relevant to humans based on the most defensible biological rationale, for example using comparative pharmacokinetic data. In the absence of a clearly most relevant species, however, U.S. EPA generally chooses the critical study and species that shows an adverse effect at the lowest administered dose. This is based on the assumption that, in the absence of data to the contrary, humans may be as sensitive as the most sensitive experimental animal species.

In the absence of adequate human data U.S. EPA generally considers a "complete" database, that is, complete for the purpose of calculating a RfD for noncancer health effects, to be composed of:

- two adequate mammalian chronic toxicity studies by the appropriate route in different species;
- one adequate mammalian multi-generation reproductive toxicity study by an appropriate route; and
- two adequate mammalian developmental toxicity studies by an appropriate route in different species.

An adequate study is one which tests a sufficient number of animals of both sexes at two or more nonzero dose levels and identifies a NOAEL and LOAEL. The determination of study adequacy rests on professional judgment. A detailed discussion of the factors to be considered when evaluating the adequacy of a database and a study can be found in U.S. EPA (1994).

Uncertainty factors (UFs) are reductions in the dose rate or concentration to account for areas of scientific uncertainty inherent in most toxicity databases. The choice of appropriate uncertainty and modifying factors reflects a case-by-case judgment by experts and should account for each of the applicable areas of uncertainty and any nuances in the available data that might change the magnitude of any factor.

Typically, U.S. EPA uses uncertainty factors to account for five areas of uncertainty. The UF for human variability (designated as H) is intended to account for the variation in sensitivity among the members of the human population. The UF for experimental animal-to-human extrapolation (designated as A) is intended to account for the extrapolation from animal data to the case of humans and is considered to have components of both toxicokinetics and toxicodynamics. The subchronic-to-chronic UF (designated as S) is intended to account for extrapolating from less than chronic levels to chronic levels. The UF for LOAEL-to-NOAEL extrapolation (designated as L) is applied when an appropriate NOAEL is not available to serve as the basis for a risk estimate, and extrapolation from an experimental LOAEL is necessary. Database completeness (designated as D) is intended to account for the inability of any single study to adequately address all possible adverse outcomes. U.S. EPA currently uses an additional factor, referred to as a modifying factor (MF), as an occasional adjustment in the estimation of an RfD to account for areas of uncertainty not explicitly addressed by the usual factors.

The traditional default value of 10 has been generally used for each of these UFs; U.S. EPA, however, through experience of calculating thousands of RfDs has developed criteria for reducing UFs (generally to a half-log value of 3, or 1), when data warrant. U.S. EPA also recognizes the potential overlap between UFs and attempts to accommodate this. A recent publication discusses the use of factors other than default based on these criteria (Dourson et al., 1996).

The equation that U.S. EPA uses to determine the value of the RfD is:

RfD = NOAEL or LOAEL(mg/kg/day) ÷ (UF x MF)

where:

NOAEL = No Observed Adverse Effect Level
LOAEL = Lowest Observed Adverse Effect Level
UF = Uncertainty Factor
MF = Modifying Factor.

Finally, U.S. EPA provides a statement of confidence in their noncancer risk estimates (Barnes and Dourson, 1988; Dourson, 1994). High confidence indicates a judgment that additional toxicity data are not likely to change the RfD. Low confidence indicates that at least a single, well-conducted, subchronic mammalian bioassay by the appropriate route is available. For such a minimum database, the likelihood that additional toxicity data may change the RfD is greater. Medium confidence indicates a judgment somewhere between high and

low. Example of confidence statements for RfDs can be found on U.S. EPA's IRIS (U.S. EPA, 1996).

2. Hazard Identification

2.1 Review of Relevant Data

Perchlorate was used until the mid-1960's in the treatment of people who are hyperthyroid because of Graves' disease. Many studies have examined the effects of perchlorate in Graves' patients but few have studied the effects in normal humans. The studies that were conducted in normal humans do not look at long-term exposure to perchlorate. Long-term studies in animals, clearly show thyroid toxicity at high doses; although, generally, these studies did not examine targets other than the thyroid. In summary, the perchlorate database defines well the mechanisms by which perchlorate acts on the thyroid but provides little information on the dose-response of perchlorate or on the likely effects in normal humans after chronic exposure to low doses. This had lead to investigation of the effects that lower doses of perchlorate have on the pituitary-thyroid axis.

2.1.1 Toxicity Data in Humans

The thyroid gland appears to be the principal target organ for perchlorate toxicity in humans. In humans, the only other effects seen are hematological effects in Graves' patients at doses 100-fold higher than those needed to affect iodide concentration in the thyroid. However, experts in the field have suggested that these hematological effects are a hypersensitivity reaction and unrelated to the effects that perchlorate have on iodine balance in the thyroid.

In normal humans, the synthesis and secretion of thyroid hormones are controlled by a feedback mechanism involving the production of thyroid stimulating hormone (TSH) by the anterior pituitary. Iodide levels in the thyroid also play a role in the control of thyroid hormone levels. TSH causes the thyroid to initiate new hormone synthesis. Its production in the pituitary gland responds to blood levels of T3 and T4. When circulating levels of T3 and T4 decrease, the production of TSH in the pituitary increases. Increased levels of circulating T3 and T4 lead to decreased pituitary production of TSH. In vitro studies of iodide transport in sheep thyroid tissue slices (Wolff and Maurey, 1962) and phospholipid vesicles (Saito et al., 1983) have confirmed that perchlorate competitively inhibits iodide transport into the thyroid. A summary of the human studies of perchlorate is presented in Table 1.

Table 1. Human Studies of Perchlorat

Study	Duration/ Number of Subjects	Doses mg/kg-day	Effects	Notes	Uncertainty Factors	RfD mg/kg-day
Stanbury and Wyngaarden (1952)	Single dose 3 subjects	0 0.04 0.14 1.4 LOAEL	Release of iodine from thyroid. Inhibition of iodine uptake by thyroid.	Graves' disease patients.	3L 3S 3H 3D 100	1E-2
Connell (1981)	22 years 1 subject	3 LOAEL	No adverse effects with clinical control of hyperthyroidism	Graves' disease patients	3L 3H 3D 30	1E-1
Godley and Stanbury (1954)	28 weeks 24 subjects	0 8.6 LOAEL	Gastrointestinal irritation in 2/24 patients. Decrease of iodine uptake by thyroid	Graves' disease patients		[a]NA
Crooks and Wayne (1960)	unknown duration 35 subjects 165 subjects 10 subjects 40 subjects	 8.6 LOAEL 14 21 28	Skin rash, nausea at 8.6-14. Also agranulocytosis at 21	Graves' disease patients		[a]NA
Morgan and Trotter (1960)	2-3 weeks 180 subjects 67 subjects	 6-14 LOAEL 17-28	Skin rash, sore throat, GI irritation, lymphadenopathy (3% in low dose, 18% in high dose)	Graves' disease patients		[a]NA
Hobson (1961)	33 weeks 1 subject	9-11 FEL	Fatal aplastic anemia	Graves' disease patients		[a]NA
Johnson and Moore (1961)	3 months 1 month 1 subject	14 FEL 9 FEL	Fatal aplastic anemia	Graves' disease patients		[a]NA

Study	Duration/ Number of Subjects	Doses mg/kg-day	Effects	Notes	Uncertainty Factors	RfD mg/kg-day
Fawcett and Clark (1961)	6 months 2 months 1 subject	9 FEL 6 FEL	Fatal aplastic anemia	Graves' disease patients		[a]NA
Krevans et al. (1962)	2 weeks 10 weeks 4 months 1 subject	11 FEL 9 FEL 6 FEL	Fatal aplastic anemia	Graves' disease patients		[a]NA
Gjemdal (1963)	4 months 1 subject	6-9 FEL	Fatal aplastic anemia	Graves' disease patients		[a]NA
Barzilai and Sheinfeld (1966)	2 months 2 subjects	14 FEL	Fatal aplastic anemia and fatal agranulocytosis	Graves' disease patients		[a]NA
Burgi et al. (1974)	8 days 5 subjects	0 9.7 LOAEL	Release of iodine from thyroid	Healthy volunteers	3L 3S 10H 3D 300	3E-2
Brabant et al. (1992)	4 weeks 5 subjects	12 LOAEL	Decrease in thyroid iodine concentration, free T4, thyroglobulin, and TSH (Follow up study shows increase thyroid volume at same dose)	Healthy volunteers pretreated with iodine for 4 weeks before perchlorate exposure		

* Table is ordered in roughly increasing dose.

Note a: This may not be an appropriate study on which to base an RfD because the doses are within the range of those that cause frank effects in Graves' patients. However, this frank toxicity may be caused by susceptibility unrelated to perchlorate exposure. See text for discussion.

2.1.1.1 Studies in Patients with Graves' Disease

Potassium perchlorate has been used to treat Graves' disease in humans and most of the data on perchlorates effects on humans are in patients with this disease. Graves' disease is an autoimmune disorder in which patients carry immunoglobulins in their blood which bind to the TSH receptors on thyroid cells and act like TSH to stimulate DNA synthesis and cell divisions leading to a hyperthyroid state. Symptoms of the disease include increased synthesis and secretion of iodide containing hormones into the blood by the thyroid gland, thyroid gland enlargement, increased basal metabolism and loss of weight. Perchlorate inhibits the excessive synthesis and secretion of thyroid hormones by inhibiting the accumulation of iodide in the thyroid.

Stanbury and Wyngaarden (1952) evaluated perchlorate in patients with Graves disease and found that perchlorate caused the discharge of iodine accumulated in the thyroid and blocked the uptake of iodine into the thyroid. Within 30 minutes of administration, a single dose of 100 mg potassium perchlorate caused the nearly complete release (~80%) of I^{131} from the thyroids of 8 Graves' disease patients previously treated with tracer amounts of I^{131} and 1-methyl-2-mercaptoimidazole (MMIA)*. A single dose of 10 mg perchlorate appeared to cause about a 50% release of accumulated iodine and the authors reported that perchlorate doses as low as 3 mg caused detectable, but incomplete, release of iodide from the thyroid (data for doses less than 10 mg were not presented). In addition, Stanbury and Wyngaarden (1952) reported that the uptake of tracer levels of I^{131} into the thyroid glands of patients with Graves' disease was markedly inhibited for as long as 6 hours when 100 mg of potassium perchlorate was given orally 1 hour prior to administration of the tracer.

* 1-methyl-2-mercaptoimidazole (MMIA) is an antithyroid agent that inhibits incorporation of iodide into thyroid hormone molecules. Pretreatment with MMIA ensured that any I^{131} accumulated in the thyroid was not used to produce thyroid hormone.

Inhibition of iodine occurred both in two patients treated with MMIA and three patients without MMIA treatment.. The authors state that no toxic effects were encountered in any of these patients who were given no more than three doses for a total of not more than 600 mg potassium perchlorate. . This study identifies a definitive LOAEL of 1.4 mg/kg/day[b] for complete release of iodine from the thyroid. Since it is not clear what degree of iodine release constitutes an adverse effect, we have not designated a NOAEL for this study.

Godley and Stanbury (1954) report using potassium perchlorate to treat 24 patients with Graves' disease. Patients were treated with 600-1200 mg/day for at least 11 weeks and as long as 45-52 weeks. Two patients developed gastrointestinal problems that were assumed to be due to perchlorate treatment. In one patient, these effects occurred at 600 mg/day, but the dose which the other patient received is not specified. Other side effects of antithyroid agents, such as hematological changes, liver damage, or skin rash, were not observed. This study suggests a LOAEL of 9 mg/kg/day.

Crooks and Wayne (1960) observed one case of skin rash and three cases of nausea (2%) among 35 patients treated with 600 mg/day (9 mg/kg/day) and 165 patients given 1,000 mg/day (14 mg/kg/day). In another group of 10 patients given 1500 mg/day (21 mg/kg/day) and 40 patients given 2000 mg/day (29 mg/kg/day), five cases of skin rash, two cases of nausea and one case of agranulocytosis occurred (16%). Leukocyte counts returned to normal in the patient with the agranulocytosis when perchlorate treatment was stopped. The length of treatment in unclear but appears to have been up to 22 weeks. The authors report the "time to cure" for perchlorate of approximately 9 weeks. The authors also report 1 of 12 infants born of mothers given 600 to 1000 mg/day, was born with a very slightly enlarged thyroid which returned to normal size in six weeks; no other abnormalities were noted. This study defines a LOAEL between 9 and 14 mg/kg/day.

Morgans and Trotter (1960) reported that 3% of 180 patients treated with 400 to 1,000 mg/day (6 to 14 mg/kg/day) potassium perchlorate and 18% of 67 patients treated with 1,200 to 2,000 mg/day (17 to 29 mg/kg/day) displayed a variety of adverse reactions including skin rash, sore throat, gastrointestinal irritation and lymphadenopathy. Reactions occurred within 2-3 weeks of drug administration. This study defines a LOAEL between 6 and 14 mg/kg/day.

[b] Unless otherwise indicated, for human studies in which the actual body weight of the subjects was not reported, the dose in mg/kg/day was calculated assuming a body weight of 70 kg. Thus a dose of 100 mg/day ÷ 70 kg is 1.4 mg/kg/day.

Connell (1981) reported a case study of a single Graves' disease patient who was treated with potassium perchlorate at 200 mg/day (3 mg/kg/day) for 22 years without any indication of adverse side effects. This dose level provided sufficient clinical control of the hyperthyroidism.

Between 1961 and 1966, the occurrence of severe hematological side effects in patients receiving long-term potassium perchlorate treatment for Graves' disease led to a decreased use of potassium perchlorate as a therapeutic agent. Several authors (Hobson, 1961; Johnson and Moore, 1961; Fawcett and Clark, 1961; Krevans et al., 1962; and Gjemdal, 1963) report case studies where a single patient suffered fatal aplastic anemia after treatment with doses ranging from 6 to 14 mg/kg/day. The duration of treatment ranged from 3 months (Johnson and Moore, 1961) to 8 months (Hobson, 1961). In all cases, patients were started out at the high end of the treatment range for a period of time and then were reduced to the lower end of the treatment range after the appearance of side effects. In two cases (Hobson, 1961 and Gjemdal, 1963) patients had co-exposures to other drugs. Other case reports are available which report nonfatal agranulocytosis in patients treated with 14 mg/kg/day for 12 days (Southwell and Randall, 1960) or 3 months (Sunar, 1963). Barzilai and Sheinfeld (1966) report that 11% of 76 patients developed leukopenia or other unspecified side effects after treatment with 1,000 mg/day (14 mg/kg/day) for as little as 2 months. Within this group, there was one case of fatal aplastic anemia and one case of fatal agranulocytosis. These studies indicate that doses in the range of 6 to 14 mg/kg/day represent a frank effect level (FEL) inpatients with Graves' disease. There is no information to suggest that humans without Graves' disease would have a similar reaction to perchlorate (See Section 2.1.1.3).

2.1.1.2 Studies in Normal Humans

Far fewer data are available to demonstrate the effects of perchlorate in normal, healthy individuals. In the available studies, exposure to perchlorate was short - just a few days to 4 weeks. Burgi et al. (1974) examined the effects of perchlorate on the secretion of endogenous iodine by the normal human thyroid gland. Five healthy volunteers received tracers of I^{125}-iodide and I^{131}-thyroxine for 17 days followed by 600 mg/day perchlorate (9.7 mg/kg/day, based on actual reported average body weight of 61.8 kg) perchlorate for 8 days. Urine and serum were analyzed for I^{125} and I^{131} to determine if perchlorate can cause the discharge of endogenous as well as exogenous iodide from the thyroid. Results show that this dose of perchlorate was sufficient to completely block iodide uptake by the thyroid. In addition, perchlorate caused a 65% increase in excretion of non-thyroxine iodine over background. The authors attributed this increase to additional secretion of endogenous iodide from the thyroid.

Treatment with carbimazole plus perchlorate caused a further increase in the secretion of non-thyroxine iodine, suggesting that perchlorate causes only a partial, not complete, release of endogenous iodide. This study defines a minimal LOAEL of 9.7 mg/kg/day.

Brabant (1992) administered potassium perchlorate to healthy volunteers as a means to study changes in TSH concentration and release in response to a decrease in iodine supply to the thyroid. During the first 4 weeks of the study, the volunteers were given 200 ug/day iodine. After iodine supplementation was discontinued, the volunteers were orally administered 900 mg/day of potassium perchlorate for four weeks to induce a state of iodine depletion. At the end of the 4-week perchlorate treatment, levels of thyroid hormones were measured. Although perchlorate treatment had no effect on thyroid volume or levels of T3 and T4, intrathyroidal iodine concentration was significantly decreased, serum levels of TSH were significantly decreased, and serum levels of thyroglobulin were almost doubled. The authors speculate that the decrease of TSH, which is opposite of the expected response, may be an early adaptive mechanism to the iodine deficiency induced by perchlorate. They suggest that early in iodine deficiency, the thyroid becomes more sensitive to TSH, creating a feedback mechanism that decreases TSH levels. Only as iodine deficiency becomes more prolonged do TSH levels increase. This study defines a LOAEL of 13 mg/kg/day for thyroid effects.

In a follow up study, Brabant (1994) repeated his studies with perchlorate treatment longer than 4 weeks. As a result of the longer treatment, thyroid volumes increased in all subjects, although TSH levels did not increase.

2.1.1.3 Role of Perchlorate in Autoimmunity and Hematological Effects

Treatment of Graves' disease patients with perchlorates has resulted in serious hematological effects in a small number of people. These effects include fatal aplastic anemia and agranulocytosis as well as less serious effects including reversible agranulocytosis, lympadenopathy, and leukopenia. Skin rash has also been frequently reported as a side effect of perchlorate treatment and may be related to the effects of perchlorate on the hematological system. For risk assessment purposes, several questions regarding the relationship between Graves' disease patients and normal humans must be answered.

- Will perchlorate have the same hematological effects in normal humans after prolonged exposure?

- Are Graves' disease patients uniquely sensitive to the hematological effects of perchlorate?

- By what mechanism does perchlorate cause hematological effects and are these effects related to perchlorate's effect on the thyroid?

The development of aplastic anemia is highly variable in the population and related to individual susceptibility. The data suggest that the altered immune function of Graves' disease patients renders them uniquely susceptible to these types of hypersensitivity reactions.

As described above, Graves' disease is an autoimmune disease in which patients carry autoantibodies to thyroid tissue which mimic TSH stimulation. Although cells from Graves' patients have an increased prevalence to express certain HLA (major histocompatibility complex) antigens (Robbins, 1979; Holland et al., 1991), Graves' disease is thought to be mediated by altered function of activated T lymphocytes (Holland et al., 1991; Panayi, 1995). Most Graves' patients have a lymphocytic infiltrate of the thyroid (Robbins, 1979). Holland et al. (1991) report the development of Graves' disease in a male patient eight years following a bone marrow transplant from his sister who had Graves' disease. The clinical findings support a role for circulating lymphocytes in the initiation of the disease.

While Graves' disease is the product of disrupted immune function, there is also evidence that hyperthyroidism itself alters immune function. In animals, hyperthyroidism results in diminished suppressor T cell function (Wenzel and Lente, 1984). In addition, Graves' disease patients in whom hyperthyroidism was not in control had decreased T cell counts but Graves' patients in whom hyperthyroidism was under control had normal T cell counts (Wenzel and Lente, 1984). Thus, it seems probable that thyroid hormone levels alter lymphocyte populations and properties. Also, patients with Graves' disease are likely to be more susceptible to idiosyncratic reactions to compounds which act on the immune system.

Antithyroid drugs appear to exert their effects on the hematopoietic system through an immune mechanism. Wing and Fantus (1987) reviewed the adverse effects of two antithyroid drugs, propylthiouracil and methimazole, and concluded that most reactions were related to immunologic effects of these drugs. They noted that skin rash and granulocytopenia were among the most commonly reported adverse effects of these drugs. Less commonly reported effects include aplastic anemia, leukopenia, and antibodies to insulin and glucagon. In fact, Wing and Fantus (1987) recommend that patients be instructed to report skin rash immediately as this may be an early sign of adverse immune reaction caused by

the antithyroid drugs. Although these authors did not include perchlorate in their investigation, the similarity of the effects seen after perchlorate treatment, including rash, leukopenia, agranulocytosis, and aplastic anemia, suggest that perchlorate may also act to induce an immune effect in a similar fashion.

There is a tight functional connectivity between the immune and endocrine systems, which is mediated, at least partly, by shared receptors and mediators among the systems (Kammuller, 1995). Thus, although the mechanism of perchlorate action on the hematopoietic system is not known, it is likely to be an immune reaction. Although it is possible that perchlorate may cause the hematological effects in normal humans, it appears that Graves' patients are likely to be more sensitive to this type of immune-induced adverse effect than normal humans. The underlying abnormal immunologic function in Graves' disease makes these patients more sensitive to immunologic challenges. Immunoreactivity to antithyroid drugs is another expression of the abnormal immune system in these patients (Wall et al., 1984; Wing and Fantus, 1987). Thus they are expected to have drug allergies with increased frequency (Wall et al., 1984).

2.1.2 Toxicity Data in Animals

Both short-term and long-term studies in animals have evaluated the effects of perchlorate on the thyroid. These studies established LOAELs at high doses, and they generally did not examine tissues and systems other than the thyroid. The long-term studies demonstrate that continual disruption of the thyroid-pituitary axis by perchlorate will result in the development of thyroid tumors.[c] A summary of the animal studies of perchlorate is presented in Table 2.

2.1.2.1 Short-term and Subchronic Studies

Mannisto et al. (1979) measured serum levels of TSH, T3 and T4 by radioimmunassays in groups of 5-6 male Sprague-Dawley rats weighing 180-220 grams who were exposed to potassium perchlorate in their drinking water at concentrations of 0, 10, 50, 100, or 500 mg/L for four days. Potassium perchlorate doses of 0, 1.5, 7.6, 15.3, and 76.3 were calculated assuming a body weight of 0.2 kg and a water consumption rate of 0.0305 L/day (U.S. EPA, 1987). Perchlorate produced statistically significant increases in serum TSH levels and decreases in serum T3 and T4 levels. Significant changes in all three parameters were measured in the 100 and 500 mg/L (15.3 and 76.3 mg/kg/day) dose group. In the 50 mg/L (7.6 mg/kg/day) dose group levels of T3 and T4 were significantly decreased; TSH levels were slightly increased, but the increase was not significant. At the low dose, T3, T4, and TSH levels were unchanged from controls. This study identifies a NOAEL of 1.5 mg/kg/day and a LOAEL of 7.6 mg/kg/day.

Caldwell et al.(1996) administered ammonium perchlorate in drinking water at concentrations of 0, 1.25, 5.0, 12.5, 25, 50, 125, or 250 mg/L to Sprague-Dawley rats (6/sex/group) for 14 days. The authors calculated the corresponding doses to be 0, 0.11, 0.44, 1.11, 2.26, 4.32, 11.44, and 22.16 mg/kg/day for males and 0, 0.12, 0.47, 1.23, 3.06, 4.91, 11.47, and 24.86 mg/kg/day for females. Thyroid weights were measured and thyroid hormone levels were measured using a radioimmune assay technique. Relative thyroid weights were statistically significantly increased in the two highest dose groups compared with controls. Thyroglobulin levels and TSH increased in both male and female rats in a dose-dependent manner. The TSH increase was statistically

[c] Because this assessment concerns the non-cancer effects of perchlorate, the findings of tumors in the long-term animal studies are not reported or evaluated. According to U.S. EPA's thyroid cancer policy, the development of thyroid cancer after continual disruption of the thyroid-pituitary axis is considered to be by a threshold mechanism. The implications of this policy for quantitative risk assessment of perchlorate are discussed in Section 2.2.2.

Table 2. Animal Studies of Perchlorate*

Study	Species (n)	Duration	Doses mg/kg-day	Effects	Notes
Kessler and Kruskemper (1966)	Male Wistar rat (6-8/group)	2 years	0 (water) 1339 LOAEL	Increased absolute and relative thyroid weight. Follicular cell hyperplasia	No tissues other than thyroid examined
Pajer and Kalisnik (1991)	Female Balb/c mice (36/group)	46 weeks	0 (water) 2147 LOAEL	Increased thyroid volume, pituitary TSH, histopathologic changes in thyroid. Follicular cell carcinoma	No tissues or organ systems other than thyroid examined
Shigan (1963)	Rabbits, Rats (# and sex not specified)	9 months	0 (water?) 0.25 NOAEL 2 LOAEL 40	Increased secretion of iodine from thyroid; no effects on other organs	Other effects examined were cardiac electrical activity, liver function, and conditioned reflexes.
	Rabbits, Rats (# and sex not specified)	3 months	0 (water) 190 LOAEL	Changes in cardiac electrical activity and liver function	Study not well enough reported or translated to be useful for risk assessment
Gauss (1972)	Female NMRI mice	160 days	0 (diet) 2011 LOAEL	Increased thyroid volume and histological changes to thyroid	
Hiasa et al. (1987)	Male Wistar rats (20/group)	20 weeks	0 (diet) 81 LOAEL	Increased absolute and relative thyroid weight, serum TSH. Decreased serum T_4	No effect on body or liver weight. No other parameters examined. No histopathology.
Caldwell et al. (1996)	Male Sprague-Dawley rats (6/group)	14 days	0 (water) 0.11, NOAEL 0.44 LOAEL 1.11 2.26 4.32 11.44 22.16	Increased relative thyroid weight, increased TSH and thyroglobulin, decreased T_3/T_4	No other tissues or organs examined

Study	Species (n)	Duration	Doses mg/kg-day	Effects	Notes
	Female Sprague-Dawley rats (6/group)	14 days	0 (water) 0.12 LOAEL 0.47 1.23 3.06 4.91 11.47 24.86	Same effects as in males. Females are more sensitive.	No other tissues or organs examined
Mannisto et al. (1978)	Male Sprague-Dawley rats (5-6/group)	4 days	0 (water) 1.5 NOAEL 7.6 LOAEL 15.3 76.3	Increased TSH and decreased T_3/T_4	No other endpoints examined; no histopathology.
Brown-Grant (1966)	Female Wistar rats (11/group)	gestation days 2-8	0 (water) 63 246	None	Developmental effects and maternal toxicity not evaluated. Only endpoint examined was the number of live litters.
Brown-Grant and Sherwood (1971)	Female Wistar rats (10/group)	gestation day 0 to 12/13	1% in water LOAEL	Decreased number of dams with implantation sites, increased maternal and pup thyroid weight	No untreated controls
Postel (1957)	Female guinea pigs	gestation day 21-48	0 (water) 740 LOAEL	Increased fetal thyroid weight	Fetuses were not examined for other developmental effects

* Table is ordered in roughly decreasing exposure duration.

significant at the 0. 47 mg/kg/day dose for females and at the 1.11 mg/kg/day dose for males. Both T3 and T4 showed statistically significant decreases; however, the T4 effect did not show a dose relationship. For T3, the decrease was statistically significant at the lowest dose, 0.12 mg/kg/day, in females and at the 0.44 mg/kg/day dose level in males. This study suggests that female rats are more sensitive than male rats to the effects of perchlorate. This study defines a LOAEL in females of 0.12 mg/kg/day. The same dose in males is a NOAEL.

Shigan (1963) administered 190 mg/kg/day in water to rabbits and white rats (number, sex and strain not identified) for 3 months. The author does not indicate if the compound was administered in drinking water or gavage with water. The animals were examined for cardiac function, liver function based on changes in serum proteins, immune function based on leukocyte phagocytosis, and adrenal function. Perchlorate at the dose tested caused a change in the EKG and a decrease in serum proteins indicating a disruption of the glycogen -forming function of the liver. The authors do not indicate if these changes were observed in both rabbits and rats. Perchlorate had no effect in the remaining tests. This study suggests a LOAEL of 190 mg/kg/day; although the study is incompletely reported and/or translated, limiting its usefulness for risk assessment.

In a second set of experiments, Shigan (1963) also treated rabbits and white rats (number, sex, and strain not identified) with 0, 0.25, 2, and 40 mg/kg/day of potassium perchlorate for 9 months. The medium for dosing was not reported. The animals were examined for cardiac function, liver function, and conditioned reflexes. In addition, uptake and discharge of iodine by the thyroid was examined. In the two highest dose groups, there was a statistically significant increase in the amount of iodine excreted from the thyroid; this increase was not observed in the 0.25 mg/kg/day dose group. The study does not indicate if the effect was seen in one or both species tested. This study suggests a NOAEL of 0.25 mg/kg/day and a LOAEL of 2 mg/kg/day for thyroid effects.

Hiasa et al. (1987) measured serum levels of T3, T4, and TSH by radioimmunassay in groups of 20 male Wistar rats administered 0 or 1,000 ppm potassium perchlorate in the diet for 20 weeks. Assuming a body weight of 0.34 kg (the average final body weight of rats treated with perchlorate alone) and a food consumption rate of 27.4 g/day (U.S. EPA 1987), an estimated dose of 80.7 mg/kg/day can be calculated. Absolute and relative thyroid weights were significantly increased compared with controls in perchlorate treated rats. No effects were seen on liver weights. T4 levels decreased slightly, but the decrease was not statistically significant. T3 levels were unchanged compared with controls. TSH levels were statistically significantly increased compared with controls. Histological exam of the thyroid revealed diffused small follicles in

perchlorate treated rats and 1 case of follicular hyperplasia. The 80.7 mg/kg/day dose is a LOAEL.

Gauss (1972) fed female NMRI mice a diet containing 0 or 1% potassium perchlorate for up to 160 days. Mice were between 50 and 60 days old at the beginning of treatment and weighed between 19 and 28 grams (average of 23.23 g). During the first two months of treatment, body weights increased about 12%; body weight data for longer treatment periods were not reported. Assuming a body weight of 23 g and a food consumption value of 4.625 g/day (U.S. EPA 1987), a dose of 2,011 mg/kg/day can be calculated. Thyroid glands were examined histologically at 10-20 day intervals through the 160 days. Thyroid volume, nuclei volume and height of epithelial follicles were increased in treated mice throughout the treatment period compared with controls. The English translation summary of the histological examinations described a progressive change in the histological appearance of the thyroid of treated mice, beginning with colloid loss, nuclei volume expansion and rising epithelium height, followed by the appearance of hyperplasia and hypertrophy of the thyroid parenchyma. At later stages of the treatment period, hyperplastic follicles, areas of adenomatic tissue, adenoma complexes and secreting cystadenomas were observed. However, no progression to malignancy was apparent. The 2,011 mg/kg/day dose is a LOAEL.

2.1.2.2 Long-term Studies

Kessler and Kruskemper (1966) provided potassium perchlorate in drinking water at a concentration of 0 or 1% to male Wistar rats for 2 years. Body weights and thyroid weights were reported for groups of 6-8 rats sacrificed after 0, 40, 120, 220, and 730 days of treatment. Thyroid glands from the animals were examined histologically. Using body weight data provided in the report to calculate a time-weighted average body weight of 0.336 and using an estimated water consumption of 0.045 L/day [calculated with the allometric equation recommended by U.S. EPA (1987)], a dose of 1339 mg/kg/day is derived. Body weights of control and treated animals were comparable throughout the experiment. In contrast, thyroid weights (both relative and absolute)were markedly increased in treated rats compared with controls at each examination interval. Histological examination of thyroids from treated rats at 40 days revealed follicular cell hyperplasia.[4] The authors characterized these changes as typical for a thyroid gland stimulated by TSH for a relatively short period of time. After 200 days of perchlorate treatment, diffusely degenerative

[4] Follicular cell hyperplasia is defined by small follicle with high epithelia and large nuclei, numerous mitoses, colloid resorption and low-grade mesenchymal reaction.

changes with fibrosis and increased colloid were observed. The authors commented that the course of the histological changes in the thyroid was similar to that produced by long-term administration of thiouracil, another antithyroid agent. The authors further reported that four of eleven rats treated with potassium perchlorate for 2 years displayed benign tumors of the thyroid gland and that 20 untreated Wistar control rats displayed no thyroid gland tumors. The 1,339 mg/kg/day dose is a LOAEL.

Pajer and Kalisnik (1991) administered 0 or 1.2% sodium perchlorate in drinking water to groups of 36 female BALB/c mice (12/group) for up to 46 weeks. Eight or 12 weeks after the beginning of the experiment, one group of treated and control mice were totally irradiated with 0.8 Gy on 5 consecutive days, at a dose rate of 1.45 Gy/minute, so that each mouse received a total of 4 Gy. Assuming a body weight of 0.0353 kg and a water consumption rate of 0.0063 L/day (U.S. EPA 1987), a dose of 2147 mg/kg/day can be calculated. Thirty animals died during the experimental period although details about the cause of death were not provided. Forty-two animals were sacrificed at 46 weeks for histological examination of the thyroid and pituitary. No other tissues were examined. Obvious treatment related histological changes were observed in the thyroid and pituitary, including thyroid follicular cell carcinoma. Immunoperoxidase staining of pituitary thyrotropic cells with antihuman TSH serum provided qualitative evidence of increased TSH production in the pituitary. Perchlorate treatment was associated with increased total volume of the thyroid gland and the distal parts of the anterior pituitary (adenohypophysis). In addition, increased average volume and increased numbers of epithelial, thyrotropic and parafollicular cells was observed. Irradiation appeared to enhance the effects of perchlorate treatment. This study identifies a LOAEL of 2147 mg/kg/day for thyroid effects.

2.1.2.3 Developmental/Reproductive Toxicity Studies

Brown-Grant (1966) examined perchlorate for its effects on pregnancy in rats. Potassium perchlorate at a 1% solution in drinking water was administered to pregnant Wistar rats from day 2 to day 8 of gestation. Average doses were reported to be 237 mg/rat/day which is equivalent to 741 mg/kg/day assuming a body weight of 0.32 kg (U.S. EPA, 1987). Birth of a live litter occurred in 8/11 treated dams compared with 7/11 of potassium chloride treated control dams. Examination of fetuses for developmental defects was not conducted. Neither the perchlorate treated dams nor the KCl controls which did not give birth displayed any visible sign of implantation in their uteri. The author concluded that 1% potassium perchlorate in the drinking water had no effect on the course of pregnancy in rats. This study identifies a free standing NOAEL of 741

mg/kg/day; although, in the absence of examination of fetuses this judgment is tentative.

Brown-Grant and Sherwood (1971) administered 1% potassium perchlorate or 0.1% potassium iodide in drinking water to pregnant Wistar rats that were also lactating. Administration began on day 0 of pregnancy and continued until day 12 or 13. Non-lactating pregnant rats were provided with 0.1% KCl or KI by similar protocol. Untreated controls were not included in the experiment. The suckling litters were removed on days 9 or 10 and all dams were killed on day 12 or 13 and examined for the number of implantation sites. There was 100% incidence of dams with implantation sites for all groups but the perchlorate treated group in which 70% of the dams had implantation sites. The number of implementation sites per dam was comparable for all groups. Thyroid weights in the perchlorate treated dams appeared to be increased compared with the chloride or iodide treated dams. Also, thyroid weights of the offspring of perchlorate treated dams was increased compared with offspring from iodide treated dams. The authors concluded that treatment with potassium perchlorate had no significant effect on blastocyst survival or the ability to implant under conditions delaying implantation (i.e., concurrent lactation). This study defines a LOAEL of ≈ 740 mg/kg/day (assuming body weights and water intakes were similar to those in Brown-Grant, 1966) for both maternal and fetal thyroid effects.

Postel (1957) reported that administration of 1% potassium perchlorate in drinking water to pregnant guinea pigs during gestation days 21 through 48 produced enlarged thyroids in the fetuses compared with thyroids of control fetuses. In contrast, perchlorate treatment did not have any effect on the thyroids in dams. Enlarged fetal thyroids also occurred when perchlorate treatment was accompanied by daily subcutaneous treatment with T3 doses as high as 32 ug/kg/day. From water intake and body weight data, the authors calculated an average daily dose to the dams of 740 mg/kg/day. The fetuses were not examined for other developmental effects. In a separate experiment, 0 or 1% potassium perchlorate was administered to nonpregnant female guinea pigs for 30, 60, or 90 days. Thyroid enlargement and hyperplasia were apparent in treated animals after 60 or 90 days of treatment. This study identifies a LOAEL of 740 mg/kg/day for fetal thyroid enlargement.

2.2 Characterization of Hazard of Perchlorate

In both humans and animals, perchlorate acts to competitively inhibit iodide accumulation in the thyroid thereby inhibiting the production of iodide-containing thyroid hormones. The short-term consequence of this action is a response by the pituitary gland to produce TSH which in turn stimulates diffuse

cell division and growth of the thyroid gland. Effects related to disturbance of the thyroid-pituitary axis have been seen in studies in humans, both Graves' patients and normal humans, and in both short-term and long-term studies in animals. Thus, disturbance of the function of the thyroid-pituitary axis appears to be the critical effect from exposure to perchlorate salts. Disturbance of the thyroid-pituitary axis leads to both noncancer and cancer effects in both humans and experimental animals.

2.2.1 Non Cancer

Experience with treatment of Graves' patients shows that repeated oral administration of 200 mg doses taken 1 to 5 times per day (3 to 14 mg/kg/day) were effective in inhibiting the excessive production of thyroid hormones and controlling other aspects of hyperthyroidism (Connell, 1981; Godley and Stanbury, 1954; Crooks and Wayne, 1960, Morgans and Trotter, 1960). However, perchlorate within a dose range of 6-14 mg/kg/day also resulted in a small number of fatal hematological effects in Graves' patients. In each of these cases, patients were treated with perchlorate at the high end of the dose range (i.e., 9 to 14 mg/kg/day) until hematological symptoms appeared; then patient's dosage was reduced to the low end of the range (i.e., 6 mg/kg/day). Thus, the threshold for these effects appears to be between 6 and 9 mg/kg/day. Because of the serious nature of these effects, the low end of the dose range (6 mg/kg/day) should be considered a Frank Effect Level (FEL).

The hematological effects of perchlorate appear to be a hypersensitivity reaction unrelated to perchlorate's effects on iodine uptake and secretion by the thyroid. The hematological effects of perchlorate may be mediated through an immune reaction such as a drug-induced autoimmune response. Since Graves' patients already have an unbalanced immune system, they are more susceptible to the hematological effects of perchlorate (Wall et al., 1987; Wing and Fantus, 1987) and thus should be considered a sensitive subpopulation for these effects.

In addition to being a sensitive population for the hematological effects of perchlorate, it has also been suggested that Graves' disease patients are the sensitive population for perchlorate's effects on iodine balance in the thyroid. Because the thyroids of Graves' disease patients are continually stimulated by antibodies which stimulate the TSH receptor, they have essentially unregulated iodine uptake. Endocrine experts have suggested that it is plausible to assume that Graves' patients will be more sensitive to the iodine blocking effects of perchlorate than normal humans (Capen, 1996; Fagin, 1996). No data are available to identify a mechanism for this phenomenon. The same phenomenon is likely to be observed in people who are iodine deficient; because their thyroids

are also under constant stimulation, they are expected to be as sensitive to perchlorate as Graves' disease patients (Capen, 1996; Fagin, 1996). Conversely, people who are hypothyroid are likely to be less sensitive to perchlorate.

Two short-term studies in normal humans support the conclusion that the target organ for perchlorate is the thyroid. Healthy volunteers dosed with 9 mg/kg/day for 8 days (Burgi, 1974) or 12 mg/kg/day for 4 weeks (Brabant 1992, 1994) both experienced a decrease in the intra-thyroidal iodide concentration of 65% and 25% respectively. A short-coming of the Brabant studies is that the volunteers were pretreated with iodine for 4 weeks prior to perchlorate treatment. It is likely that this iodine loading affected the response to perchlorate.[c] There are no studies in normal humans which demonstrate the effects of perchlorate after long-term treatment.

Animal data support the conclusion that perchlorate affects the thyroid-pituitary axis. Both short- and long-term studies found effects such as decreased T3 and T4 levels, increased TSH levels, secretion of iodine from the thyroid, and increased thyroid weights (both relative and absolute). A short-coming of the animal database is that there are few studies adequate for risk assessment which examined any tissues or systems other than the thyroid. One study, Shigan (1963) appeared to examine cardiac and liver function in addition to thyroid function. However, either because of inadequate reporting or translation from Russian, very little information is available about the methods used or the results obtained., making this study unsuitable for risk assessment purposes. Therefore, the database does not completely rule out the possibility that perchlorate has effects on systems other than the thyroid after long-term treatment.

2.2.2 Cancer

A convincing body of evidence suggests that long-term interference with the thyroid-pituitary axis can lead to thyroid follicular cell neoplasia. This phenomenon has been the subject of extensive review (Hill et al., 1989) and is summarized below. As described in Section 2.1, TSH stimulates the thyroid follicular cells to synthesize T3 and T4, which in turn inhibit the synthesis of additional TSH. Thus, high plasma levels of T3 and T4 reduce the amount of TSH produced and low levels increase the amount of TSH produced. If thyroid hormones are not produced in response to TSH, plasma levels of TSH remain

[c] According to Godley and Stanbury (1954), "No patient failed to respond [to perchlorate therapy] in time, but several responded slowly. One of these was receiving large doses of iodine just before perchlorate was begun. Presumably the thyroid of this patient was filled with iodine, which had to be exhausted before a therapeutic effect could be achieved."

high, resulting in an ongoing stimulation of the thyroid gland. This occurs following every condition which interferes with these feedback mechanisms, including iodine deficiency, thyroidectomy, or chemical disturbance.

A series of progressive morphological changes occurs in the thyroid in response to prolonged elevated levels of TSH, regardless of the nature of the stimulus causing TSH elevation (Hill et al., 1989). Initially thyroid weight remains constant although there are significant changes in thyroid morphology including resorption of colloid from the follicular cell lumen and increases in cell volume and vascularity. With continued TSH stimulation, there is a rapid increase in thyroid weight and size associated with follicular cell hyperplasia. Ultimately with continued TSH stimulation, the diffuse hyperplasia progresses to nodular proliferation of the follicular cells and eventually to benign and malignant tumors. This progression is similar regardless of the cause of thyroid insufficiency. Hill et al (1989) lists several conditions which can lead to this progression of pathology including dietary iodine deficiency, blockage of iodine into the thyroid, interference with thyroid hormone synthesis, suppression of thyroid activity by high concentrations of iodine, enhanced metabolism of thyroid hormones, and damage to the thyroid gland. It has been suggested that rats are more sensitive to these effects of increased TSH. Rats cannot withstand a sustained increase of TSH without developing tumors; just an initial rise in TSH levels will have promoting effects (Capen, 1996).

From the foregoing discussion, it is apparent that thyroid cancer induced by interference with thyroid -pituitary homeostasis is a threshold phenomenon. In fact, U.S. EPA (1996) has adopted the policy that an assumption of a threshold is appropriate for the dose-response assessment of chemicals which cause a disruption of thyroid-pituitary homeostasis and do not have genotoxic activity relevant to carcinogenicity. Perchlorate ion was cited repeatedly by U.S. EPA (1996) as an example of a chemical known to disrupt thyroid-pituitary homeostasis by acting directly on the thyroid.

The two long-term studies of perchlorate in animals (Kessler and Kruskemper, 1966; Pajer and Kalisnik, 1991) demonstrated that perchlorate induces follicular cell carcinogenesis. The shorter term studies (Gauss, 1972; Hiasa et al., 1987) indicate that carcinogenesis is preceded by the morphological changes typical of the progression induced by TSH stimulation described by Hill et al. (1989). No genotoxicity studies were located in the literature, so it is not possible to state with certainty that perchlorate does not have any genotoxic activity relevant to carcinogenicity. However, perchlorate is so clearly acting to disrupt thyroid-pituitary homeostasis that the assumption of a threshold for dose-response assessment is appropriate. Therefore, the RfD developed in the

following section is likely to be protective for both noncancer and cancer effects of perchlorate.

3. Dose Response Assessment

3.1 Choice of Critical Study

Because it is inappropriate to derive an RfD from a FEL, the clinical data which define a FEL in the range of 6-14 mg/kg/day do not provide a suitable basis for RfD derivation. For this same reason, the studies in normal humans, which were also conducted at doses which are within the range of the FEL, are also not suitable for RfD derivation. Only one long-term study in humans reported patient response to a dose less than the range of the FEL. Connell (1981) reported that a 22 year treatment with 3 mg/kg/day perchlorate effectively controlled symptoms of hyperthyroidism without any adverse side effects. However, this study is limited because only one case was reported.

The early experiments by Stanbury and Wyngaarden (1952), however, examined the influence of acute doses of potassium perchlorate at lower dosage levels. In these experiments, single 100 mg doses of potassium perchlorate (1.4 mg/kg/day) caused a complete release of iodide from the thyroid and prevented the accumulation of subsequently administered iodide for about 6 hours. Single doses as low as 3 mg potassium perchlorate (0.04 mg/kg/day) also produced a detectable, but incomplete, release of iodide. Thus, 1.4 mg/kg/day appears to be a definitive LOAEL for acute disturbance of iodide accumulation in the thyroid. It is assumed for the purposes of RfD derivation that repeated exposure to 1.4 mg/kg/day would lead to a functional disturbance of the thyroid-pituitary axis, including decreased synthesis of thyroid hormones and increased production of TSH. Exposure to lower doses would insufficiently impair iodine accumulation and would not disturb the function of the thyroid-pituitary axis.

This LOAEL is supported by the findings of the animal studies. In Caldwell et al. (1996), T3 levels in female rats were significantly decreased by a 14-day exposure to 0.12 mg/kg/day. At a dose of 0.47 mg/kg/day an effect on both T3 and TSH was observed, with T3 levels significantly decreased and TSH levels significantly increased. In addition, Mannisto et al. (1979) reported a NOAEL of 1.5 mg/kg/day and a LOAEL of 7.6 mg/kg/day based on decreased T3/T4 and increased TSH after 4 days exposure to perchlorate.

3.2 Choice of Uncertainty and Modifying Factors

The choice of uncertainty factors to be used with the appropriate critical effect of perchlorate depends on the areas of uncertainty that exist given the quality of the database.

3.2.1 Human Variability (H)

Do existing data account for sensitive individuals?

If yes, this suggests an uncertainty factor other than a default value of 10---as low as a value of 1 in some instances [see for example, the description of the uncertainty factor for nitrates on U.S. EPA's IRIS (1995) where a NOAEL of a sensitive population was used as the basis of the RfD]. Scientists familiar with this area have considered this default factor to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences among humans. Some recent work has attempted to quantify these distinctions (Renwick, 1993).

Perchlorate's critical effect in both humans and animals is disruption of the thyroid-pituitary axis. Subpopulations who may be sensitive to this effect are Graves' patients and people who are deficient in iodine intake (Capen, 1996; Fagin, 1996). About an order of magnitude difference exists between normal human LOAELs (9.7 and 13 mg/kg/day) compared with Graves' patients LOAELs (1.4 and 3.0 mg/kg/day). These differences also suggest that the Graves' patients in the Stanbury and Wyngaarden (1952) study represent a sensitive population. Thus, a reduced factor appears warranted. Since the critical study was conducted in sensitive individuals, we recommend a UF_H of 3.

3.2.2 Inter-Species Variability (A)

Do existing data allow for a quantifiable extrapolation of animal dose to the expected human equivalent dose for effects of similar magnitude? Or as is more likely the case, for NOAELs?

If yes, this suggests an uncertainty factor other than a default value of 10---with RfDs for example, a value of 3 is often used when dosimetric adjustments are used in the determination of HEC [see U.S. EPA's IRIS (1995) for numerous examples]. Scientists familiar with this area have also considered this default factor to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences between experimental animals and humans, but also recognize that some overlap with the uncertainty factor for intra-species variability exists. Some recent work has also attempted to quantify these distinctions in general (Renwick, 1993).

Since human data are used as the basis of this RfD, this factor is not needed (i.e., $UF_A = 1$). However, data suggest that rats are more sensitive to the toxicity of perchlorate than humans. Thus, if the rat LOAEL from Caldwell (0.12 mg/kg/day) or the rat NOAEL from Mannisto (1.5 mg/kg/day) are used as the basis of an RfD, a reduced UF_A might be warranted.

3.2.3 Subchronic-to-Chronic Extrapolation (S)

Do existing data allow for a quantifiable extrapolation of the critical effect after subchronic exposure to that after chronic exposure? Will NOAELs of different critical effects after subchronic and chronic exposure, differ quantitatively?

U.S. EPA has occasionally used values less than 10 (nearly always 3-fold) with less than chronic exposures when data were available to support such a reduction [for example, see the RfD for arsine on U.S. EPA's IRIS (1995)]. Scientists familiar with this area also recognize that some overlap with this factor occurs with the database uncertainty factor (see following discussion).

For perchlorate, the initial step in the progression to overt signs of toxicity is the inhibition of iodine uptake by the thyroid and the discharge of unbound iodine from the thyroid. Without this preliminary action of perchlorate, the cascade of events that lead to altered thyroid function and morphology will not occur. Therefore, short term assays that measure the initial step in the cascade of effects are likely to be as accurate predictors of toxicity as chronic assays. Some reduction in the default UF_S of 10 seems reasonable, but a reduction to 1-fold is not. Thus, a 3-fold factor was applied. This partial reduction is supported by the Connell (1981) study which showed that a 22 year exposure to perchlorate in a sensitive individual (a Graves' patient) did not result in any adverse effects.

3.2.4 Insufficient Database (D)

Do existing data allow for a reasoned judgment of likely critical effect, given that any one toxicity study is unable to adequately address all possible outcomes?

If data exist from at least five studies (two chronic standard toxicity bioassays in different species, one two-generation reproductive bioassay and two developmental toxicity studies in different species), an uncertainty factor of 1 is applied. U.S. EPA has occasionally used values less than 10 (nearly always 3-

fold) when data were available on several, but not all 5 studies [for example, see the RfD for acetaldehyde on U.S. EPA's IRIS (1995)], and factors of 10 (generally) when data were only available from a single study. Scientists familiar with this area also recognize that some overlap occurs with the subchronic to chronic uncertainty factor (discussed previously). The general solution to this problem when subchronic studies are available in two species, is to assign the uncertainty to the subchronic to chronic factor, and not to the database factor.

For perchlorate, the database consists of a wealth of knowledge about the long-term administration of perchlorate to patients with Graves' disease, chronic studies in rats (Kessler and Kruskemper, 1966) and mice (Pajer and Kalisnick, 1991); short-term studies in normal humans (Burgi, 1974 and Brabant, 1992), rats (Hiasa et al., 1987), and mice (Gauss, 1972); and acute studies in sensitive humans (Stanbury and Wyngaarden, 1952) and rats (Mannisto et al., 1979 and Caldwell et al., 1996). However, the chronic and subchronic studies are limited by the fact that they generally only examined thyroid effects. There are also three studies that examined the effects of perchlorate on dams during gestation, on pregnancy outcome, and on fetal thyroid (Brown-Grant, 1966; Brown-Grant and Sherwood, 1971; and Postel, 1957). These studies are limited because they did not examine developmental effects other than thyroid effects. No multigenerational studies are available. Thus, although there are a variety of studies, the database is missing some elements, such that some factor is stiill warranted. A UF_D of 3 is reasonable.

3.2.5 LOAEL to NOAEL (L) Extrapolation

Do existing data allow for the use of a NOAEL, rather than a LOAEL for the estimation of an RfD?

If a well-defined NOAEL does not exist, a factor of 10 is often used. However, U.S. EPA has often used values less than 10 (nearly always 3-fold) when data suggest that the LOAEL is for a minimally adverse effect, since the hypothesized NOAEL would likely be closer to this LOAEL then to a LOAEL with greater severity. For example, compare the RfDs for acrylonitrile and 1,2 epoxybutane on U.S. EPA's IRIS (U.S. EPA, 1996). The former RfD uses a 3-fold factor with degeneration and inflammation of nasal respiratory epithelium; the later RfD uses a 10-fold factor with more severe degenerative lesions of the epithelium.

The principal study identifies a LOAEL (1.4 mg/kg/day) for a biochemical endpoint that serves as a precursor to a presumed clinical disease (i.e., hypothyroidism) in normal individuals. At this dose, however, there are no overt

signs of toxicity. Signs of toxicity in humans, including skin rash, gastrointestinal problems, and increased thyroid volume, occur at dose levels of about 6 mg/kg/day or higher. Therefore, the LOAEL of 1.4 mg/kg/day appears to be a minimal LOAEL which suggests that a full 10-fold UF for this area of uncertaintyis not needed. A UF_L of 3 is appropriate here.

3.2.6 Modifying Factor

A modifying factor is not considered necessary with this database. This is because the outstanding uncertainties can be adequately addressed with the standard factors. U.S. EPA only occasionally uses a modifying factor; for example, see the RfD for methyl ethyl ketone on U.S. EPA's IRIS (1995). The default value of 1 is appropriate for perchlorate.

3.2.7 Composite Uncertainty and Modifying Factors

The composite uncertainty factor to use with a given database for developing RfDs is a case-by-case judgment by experts, and should be flexible enough to account for each of the applicable five areas of uncertainty and any nuances in the available data that might change the magnitude of any factor. U.S. EPA describes its choice of composite UF and subcomponents for individual assessments on its IRIS database (U.S. EPA, 1996). For perchlorate, the recommended overall uncertainty factor is 100.

3.3 Determination of the RfD

Our proposed RfD is 1E-2 mg/kg/day. This value is determined from the following equation using parameters previously described.

$$RfD = (LOAEL) \div (3_H \bullet 1_A \bullet 3_S \bullet 3_D \bullet 3_L \bullet 1_{MF})$$

$$= 1.4 \text{ mg/kg/day} \div 100$$

$$= 0.014 \text{ mg/kg/day, rounded to 1E-2 mg/kg/day.}$$

where:

3_H is a 10-fold factor, applied to account for the uncertainty inherent in the differences in human sensitivity

1_A, an interspecies UF was not needed.

3_S is a 3-fold factor to account for the uncertainty in extrapolating from short-term results to chronic exposure.

3_D is a 3-fold factor to account for the uncertainty due to an incomplete database.

3_L is the factor to account for extrapolation from a LOAEL to a NOAEL.

1_{MF}, a modifying factor different than 1-fold was not needed.

3.4 Confidence in the RfD

Confidence in the critical studies is medium-to-low because these studies examined only short-term manifestations of the perchlorate critical effect. However, while Stanbury and Wyngaarden only examined the initial step in disruption of the thyroid-pituitary axis (inhibition of iodide uptake by thyroid), Caldwell et al. (1996) reported a further step in the critical effect, that is decrease of thyroid hormone levels with accompanying increase in TSH. Confidence in the database is medium-to-low. The effect of chronic exposure to low doses of perchlorate has not been adequately examined in either normal humans or animals. In addition, the database lacks adequate developmental and multigenerational studies. Reflecting confidence in the principal studies and the database, confidence in the RfD for potassium perchlorate is medium-to-low.

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Early Adaptation of Thyrotropin and Thyroglobulin Secretion to Experimentally Decreased Iodine Supply in Man

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Five healthy male volunteers (aged 25 to 28 years) were studied both after 4 weeks of treatment with 200 μg iodine/d orally (PO) and following experimental iodine depletion by treatment with 3 × 300 mg perchlorate/d PO over a 4-week period, in an attempt to better define the early adaptive responses to an alteration in iodine supply in thyroid function. Intrathyroidal iodine, serum triiodothyronine (T_3), free T_3 (FT_3), thyroxine (T_4), free T_4 (FT_4), reverse T_3 (rT_3), thyroxine-binding globulin (TBG), thyroglobulin (Tg), and thyrotropin (TSH) levels (10-minute sampling over 24 hours) were measured at the end of iodine administration and at the end of perchlorate treatment. Thyroid volume was determined by sonography, and iodine content was determined by fluorescence scintigraphy. TSH pulses were analyzed by computer-assisted programs. Comparing both experimental situations, perchlorate treatment significantly reduced intrathyroidal iodine concentration (4.9 ± 1.3 to 3.9 ± 1.2 nmol/mL, $P < .05$), but thyroid volume and total serum T_4, T_3, FT_3, and TBG levels were not altered. Mean 24-hour serum TSH levels (1.8 ± 0.2 to 1.0 ± 0.3 mU/L, $P < .001$), amount of TSH secreted/pulse (0.5 ± 0.1 to 0.3 ± 0.1 mU/L, $P < .001$), and FT_4 levels (15.7 ± 1.7 to 14.3 ± 1.4 pmol/L, $P < .005$) were significantly diminished, whereas Tg levels (18.6 ± 10.0 to 35.1 ± 14.0 ng/mL, $P < .01$) were significantly increased. Thyroid-specific antibodies were normal and were not altered by treatment. These data suggest a higher sensitivity of the thyroid to TSH in the early adaptation to iodine depletion; thus, less TSH is sufficient to maintain normal thyroid function.

THE INFLUENCE OF dietary iodine deficiency on thyroid growth and goiter formation has been thoroughly documented,[1] but the mechanisms involved in this pathophysiological adaptation are still the subject of considerable controversy. Thyrotropin (TSH), via cyclic adenosine monophosphate (cAMP)-dependent pathways, has been shown to directly stimulate specific thyroid functions such as iodine trapping, thyroid peroxidase, and thyroglobulin (Tg) gene expression.[2-4] In many species, including humans, TSH directly mediates thyrocyte proliferation and differentiation, but these effects have been disputed in other species.[5] Severe iodine deficiency decreases thyroid hormone formation, and it has been argued that an accompanying increase in circulating TSH serum levels stimulates goiter growth.[6-8] This pathophysiological link between thyroidal iodine supply and TSH-mediated thyroid proliferation has been the basis of the treatment of endemic goiter with thyroid hormones. However, an increased TSH secretion has never convincingly been demonstrated in patients with endemic goiter grades I and II, suggesting that the increase in TSH serum levels occurs late in the disease.[7,9,10] Bray[11] explained this discrepancy by an increased sensitivity of the thyroid to TSH in iodine deficiency. With the recent discovery that TSH is released in pulses,[12,13] this increased sensitivity may, as an alternative hypothesis, be explained by changes in the pattern of pulsatile TSH release. Iodine deficiency may stimulate thyrocyte proliferation not by an increase in circulating serum levels of TSH, but by altering the pattern of pulsatile TSH release. In the present study, we tested healthy male volunteers for potential early changes in the temporal pattern and absolute serum concentration of TSH in response to a decrease in iodine supply to the thyroid.

SUBJECTS AND METHODS

Five healthy male subjects (aged 25 to 28 years) participated in the study after giving written informed consent. Special care was taken to ensure normal thyroid function by measuring total and free thyroxine (T_4 and FT_4) and triiodothyronine (T_3 and FT_3) (Corning, Giessen, Germany), and by determination of thyroid-specific antibody levels (thyroid peroxidase antibodies, antithyroglobulin antibodies, and TSH-receptor antibodies; Henning, Berlin, Germany). Nodular thyroid changes were excluded by high-frequency sonography (7.5 MHz), and thyroid size was evaluated by ultrasound. During the first 4 weeks of the study, 200 μg/d iodine (Jodid 100, Merck, Darmstadt, Germany) was administered orally (PO), and at the end of this period blood was sampled under standardized conditions (sleep occurred between 12:00 AM and 7:00 AM, meals were at fixed times) every 10 minutes over 24 hours. On the following day, intrathyroidal iodine content was determined as previously described[14] by fluorescence scintigraphy (Fluorescence Scanner System 4202, EG&G Instruments, München, Germany; mounted on a Picker Magnascanner 300, Picker, Germany) using americium 241 as the source of radiation.

After iodine supplementation was discontinued, the subjects were treated with 3 × 300 mg perchlorate/d PO (Irenat, Tropon, Köln, Germany) to induce a state of iodine depletion. At the end of a 4-week period of iodine depletion, the initial 24-hour blood-sampling protocol and the evaluation of hormone levels, thyroid-specific antibody titers, intrathyroidal iodine content, and thyroid size were repeated.

T_3, T_4, FT_3, FT_4, and thyroxine-binding globulin (TBG) serum levels were measured as a mean of six serum samples obtained in 4-hour intervals by commercially available radioimmunoassay kits (Corning, Giessen, Germany; Behring, Marburg, Germany). Serum reverse T_3 (rT_3) concentrations were measured in the same serum samples, as previously described.[15] Tg serum levels were determined in 30-minute intervals, and in each 10-minute sample TSH was measured by our previously described method,[16] using commercially available immunometric kits (Henning, Berlin, Germany). Both rhythms of an individual were tested in the same set of

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assays. The maximal intra-assay or inter-assay coefficient of variation of all assays was 8.7%.

To determine pulsatile TSH release, a recently described computer-assisted program for pulse analysis, DESADE, was used, with a calculated rate of false-positive pulses of less than 1%.[16] Cross-correlation between TSH and Tg rhythms, using 30-minute intervals for both hormones, was performed using a computer-assisted program.[17] Statistical evaluation was performed by paired Student's *t* test; values are presented as the mean ± SD.

RESULTS

Mean thyroid volume in the volunteers was not significantly different at the end of the iodine supplementation period and following perchlorate treatment. In contrast, intrathyroidal iodine concentration was significantly decreased by iodine depletion. The circulating serum concentrations of thyroid hormones were not significantly altered by either treatment. Only serum FT_4 levels decreased slightly but significantly. No changes were seen in T_3/T_4 or FT_3/FT_4 ratios, thyroid-specific antibody titers, or TBG serum levels (Table 1).

Serum levels of Tg almost doubled following iodine depletion, and this effect was visible during the entire 24-hour period (Fig 1, Table 1). Data analysis by both computer-assisted programs showed no pulsatile release pattern of Tg, and no clear circadian changes of Tg were found. The pattern of TSH secretion during iodine supplementation showed the expected pulsatile and circadian variations (Table 2). Following perchlorate treatment, mean serum levels of TSH were significantly diminished, but the circadian pattern was still present (Fig 1). The analysis of the pulsatile release pattern demonstrated a significant reduction in pulse amplitude in each rhythm, but the number and distribution of TSH pulses remained unchanged (Table 2). No significant cross-correlation between Tg and TSH rhythms could be detected.

DISCUSSION

The importance of iodine deficiency for goiter formation is undisputed. TSH may mediate these effects by its interaction with iodine uptake and organification and its role in thyroid growth regulation. However, the pathophysiological changes in TSH occurring during the transition from normal dietary iodine supply to the supply expected in alimentary iodine deficiency are not well investigated. In-vitro and in-vivo test situations in animal models and in man generally focus either on the TSH-dependent thyroid function following iodine exposure that is 100-fold to 1,000-fold higher than expected, when changing from nor-

Table 1. Comparison of Thyroid Characteristics at the End of 4 Weeks' Supplementation With 200 µg Iodide/d PO and 4 Weeks' Iodine Depletion With 3 × 300 mg Perchlorate/d in Five Healthy Male Volunteers

	Iodide	*p*	Perchlorate
Thyroid volume (mL)	19.4 ± 7.2	NS	21.4 ± 8.8
Thyroidal iodide (mmol/mL)	4.8 ± 1.3	< .05	3.8 ± 1.2
T_3 (nmol/L)	2.4 ± 0.2	NS	2.4 ± 0.4
T_4 (nmol/L)	91.4 ± 10.3	NS	90.1 ± 10.3
FT_3 (pmol/L)	7.2 ± 1.5	NS	6.7 ± 0.8
FT_4 (pmol/L)	15.7 ± 1.2	.005	14.3 ± 1.4
rT_3 (nmol/L)	15.8 ± 4.7	NS	15.5 ± 4.6
Tg (ng/mL)	18.6 ± 10.8	.01	35.1 ± 14.8
TBG (µg/mL)	16.2 ± 1.3	NS	16.1 ± 2.8

NOTE. Results are means ± SD.

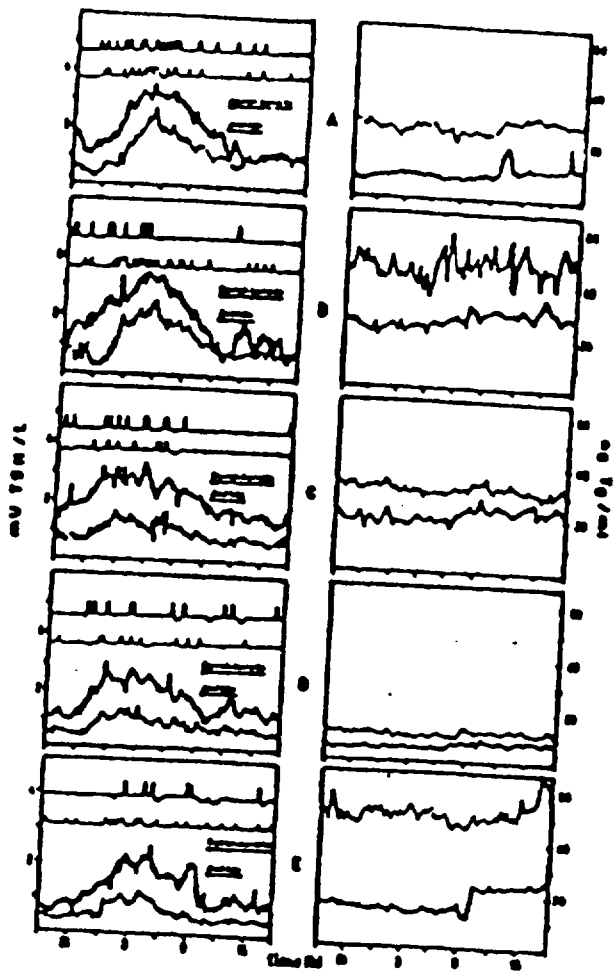

Fig 1. Individual pattern of TSH and Tg secretion in five healthy volunteers (A-E) following 4 weeks of treatment with 200 µg iodide/d PO (——) and perchlorate 3 × 300 mg/d PO (— —). Blood sampling occurred every 10 minutes for TSH and every 30 minutes for Tg. The location of TSH pulses with the DESADE program is graphically shown in the upper part of each individual graph.

Table 2. Comparison of TSH Serum Levels and Characteristics of Pulsatile TSH Secretion at the End of 4 Weeks' Supplementation With 200 µg Iodide/d PO and 4 Weeks' Iodine Depletion With 3 × 300 mg Perchlorate/d in Five Healthy Male Volunteers

	Iodide	*p*	Perchlorate
Mean TSH serum levels (mU/L)	1.8 ± 0.3	.001	1.0 ± 0.1
Number of TSH pulses/24 h	11.4 ± 4.8	NS	14.5 ± 6.1
Amount of TSH secreted/pulse (mU/L)	0.5 ± 0.1	.001	0.3 ± 0.1
Temporal distribution of pulses			
8:00 PM to 4:00 AM	6.3 ± 4.8	NS	8.0 ± 2.6
4:00 AM to 12:00 PM	3.3 ± 1.3	NS	3.9 ± 1.8
12:00 PM to 8:00 PM	1.7 ± 0.9	NS	2.6 ± 1.8

NOTE. Results are means ± SD.

mal to iodine-deficient locations, or during a blockage of iodine organification severe enough to lead to hypothyroidism.[4,5,16,22] In our study, a high-normal dietary iodine supply to the thyroid was achieved by supplementation of iodine to the mildly deficient intake in the region, leading to a daily iodine intake approximately equivalent to that in the United States. This was compared with a mild experimental iodine deficiency elicited by perchlorate treatment and led to a pronounced decrease in TSH serum levels. These changes are in parallel to findings in rats undergoing decreased iodine intake that initially show no increase in levels of TSH,[20] whereas a more intense iodine deficiency leads to a continuous increase in TSH serum levels.[19,20] Comparative epidemiological studies in humans living in regions with mild-to-moderate dietary iodine deficiency and regions with normal iodine supply, either in Italy or northern Europe, showed a comparable reduction in TSH serum levels in iodine deficiency similar to our findings.[4,23] Development of thyroid autonomy has been suggested as the mechanism responsible for this reduction in serum TSH levels[21,23]; this interpretation is not supported by our data. Development of autonomy within 4 weeks in healthy subjects seems to be unlikely. The unchanged serum levels of total thyroid hormones and the slight decrease in FT_4 levels may be a very early sign of insufficient thyroid hormone synthesis. Our findings instead point toward a higher sensitivity of the thyroid to the effects of TSH under conditions of iodine depletion. By this mechanism, TSH would more effectively release thyroid hormones, and this would lead to a reduction of circulating serum TSH levels via negative feedback. Our recent investigation of 24-hour TSH rhythms in a group of nine patients with diffuse goiter grade I, and in one case grade II, supports this finding, as TSH serum levels in these patient groups were similarly reduced compared with healthy controls.[24]

The exact mechanism of iodine interference with thyroid function is unknown. It has been shown that a high iodine supply inhibits TSH effects on cAMP formation.[18,22] Changes from a low to high-normal iodine supply in the rat, in the absence of measurable changes in thyroid hormone or TSH plasma concentrations, result in structural alterations of the thyrocyte, modification of the basolateral transfer of iodine, formation of iodolactones,[25,26] and decreases in Tg iodination and endocytotic fluxes.[27,28] Opposite effects occurring as a first adaptive mechanism of the thyroid to iodine depletion are conceivable. Furthermore, Studer et al[29] provided evidence for a heterogeneous sensitivity of thyrocytes to TSH in euthyroidism. Recruitment of previously insensitive thyrocytes to TSH action may be another mechanism of adaptation in iodine deficiency, and may lead to an increased surface area for iodine trapping. Contributing to this recruitment may be an increase in thyroid blood flow, as suggested by recent detailed studies in rats and in humans showing an inverse relationship between thyroid blood flow and iodine.[30,31]

These observations are consistent with our results showing increased serum Tg levels. A participation of a higher percentage of thyrocytes in thyroid hormone synthesis and secretion in iodine deficiency may lead to an increased release of Tg stored in thyroid follicles. Decreasing follicular stores of Tg and therefore of Tg-bound iodine in iodine deficiency, together with an increased efficacy in trapping iodine, may be an early physiological mechanism of adaptation to iodine deficiency.[22,28] Alternatively, an increased de-novo synthesis of Tg may occur, and the iodine content of this newly formed Tg may be decreased in iodine deficiency. Since there are currently no assays available to determine the iodine content of Tg in circulation, this hypothesis cannot be further evaluated. Follicular necrosis, as discussed previously,[32] may serve as another explanation in our volunteers, but the consistency of thyroid hormone levels in these healthy subjects argues against such an explanation. Finally, we could not confirm earlier findings of a direct correlation between serum Tg levels and thyroid weight, serum thyroid hormone concentrations, or the T_3/T_4 ratio.[33]

Computer-assisted analysis of the pattern of TSH secretion demonstrated that the decrease in mean 24-hour TSH serum levels was due to a reduction in pulse amplitude, but no change in the frequency of TSH pulses or their distribution was found. Since the thyroid volume of the volunteers was unaltered by treatment, these data are not necessarily in contradiction to our initial hypothesis of an altered specific temporal pattern of TSH stimulation of thyroid proliferation. The decrease in TSH serum levels may occur as a very early sign of adaptation of the thyroid to iodine deficiency, because the sensitivity of thyrocytes is increased and a greater number of thyrocytes participate in thyroid function, thus reducing the requirement of pituitary TSH stimulation. A further decrease in dietary iodine supply or a longer-lasting iodine deficiency may then alter serum concentrations of TSH and/or the pulsatile pattern of TSH secretion potentially via a decrease in thyroid hormone synthesis, subsequently stimulating thyroid growth.

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#1974

Influence of Perchlorate on the Secretion of Non-Thyroxine Iodine by the Normal Human Thyroid Gland

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Abstract. Several authors have postulated that endogenous iodide produced by the deiodination of iodotyrosines in the thyroid feeds into a different thyroidal iodide compartment than transported iodide which enters the gland from outside. One argument for the existence of two separate iodide compartments is the observation that under certain experimental conditions perchlorate completely discharges transported iodide from the thyroid, while it has no such effect on endogenous iodide. This latter observation however has not been confirmed by all studies and remained controversial. — We therefore reinvestigated the effect of perchlorate on the secretion of endogenous iodide by a new, sensitive method. Five normal volunteers received tracer amounts of iodide-^{125}I p.o. and 11 days later thyroxine-^{131}I I.V. Two days later the following serial measurements were started: serum protein-bound labelled iodine (PB^{125}I, PB^{131}I), serum total thyroxine and urinary excretion of ^{125}I, ^{127}I and ^{131}I. — In the control period the non-thyroxine iodine secretion calculated from the above measurements was 40 µg/day. Under perchlorate 200 mg three times daily this value rose significantly to 66 µg/day. Non-thyroxine iodine comprises the secreted triiodothyronine plus the secreted endogenous iodide. Assuming that the former value remained constant, our data show that perchlorate indeed discharges part, though not all, of the endogenous iodide. These data do not rule out a second iodide compartment, but they are also compatible with a simple one compartment model.

Key words: Perchlorate, thyroid, endogenous iodide, non-thyroxine iodine.

It is an accepted fact that the thyroid gland possesses two sources of inorganic iodide [1]. The first is the iodide which is accumulated from outside the cell by an active transport mechanism, the so called "transported iodide". The second sources are the iodothyrosines, which are derived by hydrolysis from thyroglobulin and rapidly deiodinated within the gland to yield the so called "endogenous iodide".

While there is general agreement that both iodide sources are used within the thyroid gland for organic iodination and therefore for hormone biosynthesis, it is still very controversial whether they actually feed into the same intrathyroidal iodide compartment [2, 3].

An important argument for the existence of two iodide compartments was based on the effect of perchlorate. This drug led to a rapid depletion of transported radioiodide in the rat, but seemed to have no effect on endogenous iodide [2, 3]. In line with this view are the observations in man that perchlorate, though preventing the active transport of iodide from outside into the gland, does not appear to discharge internal iodide [4–6].

These observations however have not remained unchallenged. In the dog, perchlorate [7–9] or thiocyanate [10] led to a massive discharge of endogenous iodide. Greer *et al.* [11] have confirmed the discharge of endogenous iodide by perchlorate in the rat, and they thought that the evidence for a second iodide pool was artifactual, a view also expressed by Wollman [12].

We have therefore considered it worthwhile to reinvestigate this controversial perchlorate effect on endogenous iodide in normal men by a very sensitive new double isotope method. The results show that perchlorate indeed leads to a small but significant increase of non-thyroxine iodine secretion which is most probably due to a discharge of endogenous iodide.

Methods

Five healthy persons served as volunteers for the investigation. The experimental set-up was adapted from the method of Wartofsky *et al.* [13–15] as detailed in a previous publication [16] and using the same material.

Experimental Protocol

Day 0: Oral intake of 80 µCi Na ^{125}I. Day 11: Intravenous injection of 30 µCi L-thyroxine-^{131}I. After a further two days allowed for equilibration of the thyroxine-^{131}I blood samples were drawn daily or at two day intervals from an arm vein. Urine samples were collected overnight during an exactly recorded time varying from 6 to 9 h. Whenever the drug regimen was changed four 6 h collections were made during one day. Time and dosage of drugs are recorded in the result section.

Measurements and Calculations

^{127}I in urine was measured by the method of Stolc and Knopp [17] in one 48 h urine sample of each subject at the beginning and in all overnight urines thereafter. The daily ^{127}I excretion in the control period was 62 µg when measured in the initial 48 h urine specimen and 59 µg when calculated from three consecutive

overnight specimens. The agreement of both values indicates that overnight urines are valid substitutes for 24 h collections. Since the subjects were eating their usual non-standardized diet, their urinary iodine excretion as expected varied from day to day [18]. To obtain more accurate values the experiment was repeated exactly in subjects No. 1 and 4, omitting the isotopes and measuring only the ^{127}I in the urine and the mean values of the two experiments were used for these two persons.

Calculations were done as described in a previous publication [16] with the exception that the fractional thyroidal uptake of radioiodide was directly obtained from the cumulative urinary isotope excretion during the 48 h following intake of ^{125}I.

Differences in mean values were statistically evaluated with student's *t*-test [19].

Results

Table 1 gives details of the experimental subjects together with the results of routine thyroid function tests.

Fig. 1 gives the radioactivity (^{125}I) of the serum iodide and the urinary excretion of ^{125}I and of ^{131}I. The latter is divided by the $PB^{131}I$ of the corresponding day to correct for variations in $PB^{131}I$ which is the source of the urinary ^{131}I. Perchlorate and later carbimazole each lead to a stepwise increase of the ^{125}I of serum which is adsorbed to the amberlite resin and mainly represents iodide[1]. The urinary excretion of ^{125}I rises exactly in parallel with the serum iodide-^{125}I. Perchlorate produces an increase of the urinary ^{131}I excretion which is identical to the one predicted on the basis of the known fractional thyroidal iodine uptake (predicted rise in ^{131}I excretion = 40.6 ± 3.8%, observed rise 42.9 ± 4.9%, the excretion under perchlorate being taken as 100%). This observation, together with other reports from the literature [6] suggests that the dose

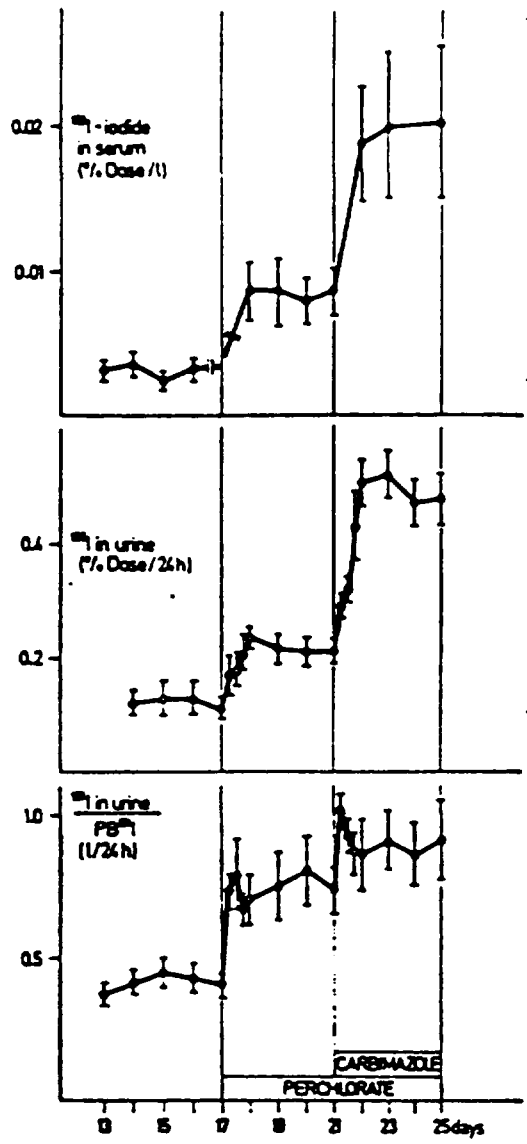


Fig. 1. Radioactivity (^{125}I) of serum iodide (top), urinary excretion of ^{125}I (middle) and ratio of urinary ^{131}I/serum protein-bound ^{131}I (bottom). The mean values with standard errors of all 5 subjects are given. Perchlorate was given in a dose of 200 mg three times daily from day 18 through to day 25. The carbimazole dose was 15 mg three times daily from day 21 through to day 25

Table 1

Subject No. (No., sex)	Age (years)	Weight (kg)	Thyroidal uptake of ^{125}I (% dose at 48 h)	Initial serum thyroxine (μg/100 ml)
1, ♀	25	60	30.7	11.2
2, ♂	24	70	41.0	12.4
3, ♀	26	51	37.0	8.6
4, ♂	27	76	40.1	11.2
5, ♀	24	52	54.0	9.9
Mean			40.56	10.66
Standard error			3.81	0.64

1 Mono- and Diiodotyrosine would also be adsorbed.

of perchlorate was sufficient to completely block iodide uptake by the thyroid. Addition of carbimazole to the drug regimen produces a further slight rise of the urinary ^{131}I which must be due to the fact that during the 7 day control period after the injection of the thyroxine-^{125}I some of the radioiodine had been taken up by the thyroid.

The thyroidal secretion of non-thyroxine iodine as calculated from the above data is depicted in Fig. 2. In the control period the mean secretion was 40 ± 6 μ

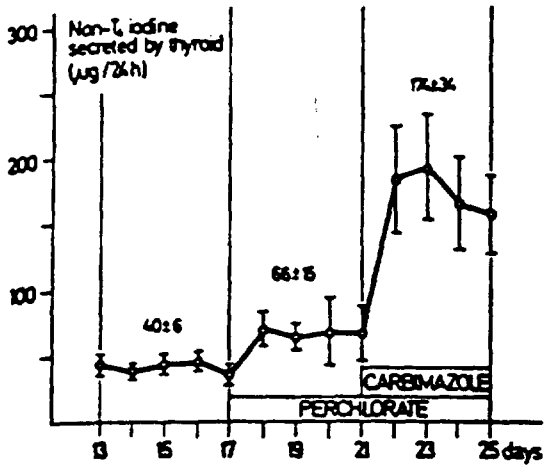


Fig. 2. Thyroidal secretion of non-thyroxine iodine as calculated from data of Fig. 1 and Fig. 2. The mean values of all 5 subjects with the standard errors are given. The abscissa and the drug treatment were the same as in Fig. 1

per 24 h (mean ± SEM). In 4 of the 5 subjects non-thyroxine iodine secretion rose significantly under perchlorate (p value <0.05 or better) and in one it decreased slightly, but not significantly. Under carbimazole plus perchlorate the secretion of non-thyroxine iodine rose further significantly in all 5 persons to a mean value 174 ± 33 μg per day. The mean values in the control period and under carbimazole are practically identical to those reported for 9 other subjects investigated under similar conditions [16].

Table 2 gives the details of the urinary excretion of ^{127}I together with a calculation of the thyroidal iodine balance. The excretion of ^{127}I rose from 60 μg in the control period to 200 μg/day in the carbimazole period (Table 2, lines b and i). An unexpected finding was that the thyroid glands of all 5 subjects were in a slightly negative iodine balance in the control period (Table 2, line f). The cause for this finding could be an error in the chemical measurement of urinary ^{127}I. We think that this is unlikely, particularly since the whole

Table 2

		Subject 1[a]	2	3	4[a]	5	Mean and SEM
Control period	a) Thyroidal radioiodine uptake (fraction of dose)	0.31	0.41	0.37	0.40	0.54	0.40 ± 0.03
	b) Urinary excretion of ^{127}I (μg/day)	59	62	53	74	52	60 ± 4.0
	c) Thyroidal secretion of thyroxine iodine (μg/day)	53	57	46	71	39	53 ± 5.4
	d) Thyroidal secretion of non-thyroxine iodine (μg/day)	29	24	54	54	42	40 ± 6.3
	e) Thyroidal absolute iodine uptake: $a \times (b+c+d)$[b] (μg/day)	44	59	57	80	72	62 ± 6.2
	f) Thyroidal iodine balance in control period: $e-(c+d)$	−38	−22	−43	−43	−9	−31 ± 6.9
Perchlorate period	g) Urinary excretion of ^{127}I (μg/day)	96	131	133	191	119	134 ± 15.4
	h) Thyroidal secretion of non-thyroxine iodine (μg/day)	60	41	120	76	34	66 ± 15.3
Carbimazole period	i) Urinary excretion of ^{127}I (μg/day)	180	153	245	269	152	200 ± 24.2
	k) Thyroidal secretion of non-thyroxine iodine (μg/day)	146	131	235	212	96	174 ± 33.5
	l) Expected rise of urinary ^{127}I from control to carbimazole period: $c+k-d$ (μg/day)	161	166	288	238	126	196 ± 29.4
	m) Observed rise of urinary ^{127}I from control to carbimazole period: $i-b$ (μg/day)	121	91	192	194	100	140 ± 22.3
	n) Observed rise of urinary ^{127}I in percent of expected rise: $100 \times m/l$ (%)	75	55	67	82	79	72 ± 4.8

[a] The ^{127}I measurements in the urine for these subjects are the mean values of two separate experiments.

[b] Assuming that iodine from endogenous sources (non-thyroxine iodine and iodine from thyroxine breakdown) is equally available to the thyroid for reutilization as exogenous iodine.

experiment was repeated in two of the subjects with excellent agreement to the first ^{127}I measurements. More likely this result must be related to findings by Dworkin [20] and Vought [21] that normal persons are in negative iodine balance most of the time if they are followed under carefully controlled metabolic ward conditions. Dworkin [20] has provided the following plausible explanation for this phenomenon: on a few days of the year a large excess of iodine is consumed, e.g. in the form of iodine-containing medications or iodine-rich food. During these days the iodine balance becames strongly positive and the thyroid gland gets "loaded" with iodine, which it slowly clears during the long periods of average iodine intake. Our subjects were instructed not to eat sea-food nor to take iodine-containing medications during the study. It is therefore not surprising that an "iodine load" with a positive balance was not observed during the 4 days of the control period.

The isotope data allow calculation of the expected rise of urinary ^{127}I from the control to the carbimazole period. By comparison of this calculated value with the value actually measured the validity of the double isotope method can be checked (Table 2, lines l and m). The data show that the double isotope method overestimates non-thyroxine iodine secretion by 28% (Table 2, line n). This systematic error is most likely due to underestimation of the specific activity ($^{125}I/^{127}I$) of thyroidal iodine, a value which cannot be measured directly in man [16]. Thus, while our ^{127}I measurements confirm the general validity of Wartofsky's double isotope method [13–15], they show at the same time that the absolute values of non-thyroxine iodine secretion it yields should be interpreted with some caution, since they may contain an appreciable error.

Discussion

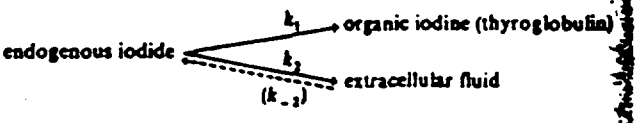
As we have outlined elsewhere [16] non-thyroxine iodine comprises between 9 to 24 μg of triiodothyronine. Quantities in excess of this value must be due to the secretion of non-hormonal iodine, which most probably represents iodide derived from the intrathyroidal deiodination of iodotyrosines.

Granted that the secretion of triiodothyronine remains constant, any increase of non-thyroxine iodine secretion must be attributed to additional secretion of endogenous iodide. Our experiments show that perchlorate indeed leads to a small but significant increase of non-thyroxine iodine secretion in 4 out of 5 subjects, and therefore, under the above premise, to an increase of the secretion of endogenous iodide of 26 μg/day. If the secretion of triiodothyronine, or that of thyroxine, had actually declined under perchlorate, then this change in endogenous iodide secretion would have been even greater. Our finding disagrees with reports by Nagataki and Ingbar [3] and by DeGroot and Bühler [6] who thought that in man perchlorate only prevented the uptake of exogenous iodide, but had no effect on the secretion of endogenous iodide. We think that the discordant results must be due to the less sensitive method used by the above investigators, who may have missed a small increase of 26 μg/day. Nicoloff [22] using a method basically similar to ours found a temporary increase of iodine release under perchlorate, lasting only about 24 h. However his method did not take into account the $PB^{131}I$ and the $PB^{125}I$ and the results cannot be fully compared. The marked rise of non-thyroxine iodine secretion and also of urinary ^{127}I excretion under carbimazole in our subjects is in good agreement with previous findings by other authors [4–6]. The results are in keeping with the view that thionamide drugs block the organification of transported as well as endogenous iodide, the latter then being secreted quantitatively.

Contrary to our finding in man perchlorate produced the secretion of *all* the endogenous iodide in the rat thyroid gland perfused *in situ* [11]. This quantitative difference may be explained by the fact that in this latter experiment the thyroids were under maximal TSH stimulation. In support of this explanation Rosenberg *et al.* [8] observed that in the dog perchlorate discharged only small amounts of endogenous iodide under basal conditions, but large quantities under TSH stimulation.

Yamada [23] has shown that perchlorate added *in vitro* at a high concentration to rat serum displaced thyroxine from albumin, the main binding protein in the rat. It is unlikely that such a displacement takes place in man where thyroxine-binding globulin is the main binding protein and where drug serum levels attained *in vivo* must be much lower. Even if such a displacement had taken place in our experiment, it would not influence our measurements of the non-thyroxine iodine secretion. The double-isotope method, thanks to the thyroxine-^{131}I injected as an internal standard, will automatically correct for any peripheral effect of drugs.

The effect of perchlorate on endogenous iodide is at first view difficult to explain. As has been pointed out by Rosenberg *et al.* [8] endogenous iodide can be disposed of either by reincorporation into thyroglobulin or by secretion, as schematically illustrated.



The fraction of endogenous iodide secreted will depend solely on the ratio of the two reaction rates k_1 and k_2. Normally k_1 is greater than k_2 and little endogenous iodide is secreted. If one takes for granted that perchlorate only blocks the influx of iodide from the extracellular fluid into the cell (a view that may be

challenged), it should theoretically have no effect on the secretion of endogenous iodide. That perchlorate *does* increase the secretion of iodide could mean either a) that it increases k_3 and that secretion of iodide is not a simple diffusion process as usually accepted, or b) that one has to consider a third reaction, namely re-entry of part of the iodide just secreted into the surrounding fluid, i.e. iodide that has left the cell, but not yet left the gland [11]. We prefer this latter explanation because it fits better with the prevailing view on perchlorate action. By the same token we think that an incomplete discharge of endogenous iodide by perchlorate may not be used as evidence for a so-called second iodide compartment. Considering the above simple model it would indeed be unexpected that perchlorate discharged all the endogenous iodide. Thus admittedly our data do not rule out the existence of a second iodide compartment, but they can be explained by a simple one compartment model.

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Effect of Perchlorate on the Human Thyroid Gland

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A FUNDAMENTAL property of the thyroid gland is that of removing iodide from the perfusing blood and concentrating it in the processes of hormone synthesis and storage. During recent years a large number of substances have been found that interfere specifically with either iodide capture (trapping) or hormone synthesis. Among those preventing the latter are clinically important drugs such as propylthiouracil and 1-methyl-2-mercaptoimidazole. These prevent the oxidation of iodide ion to iodine and its chemical attachment to tyrosyl groups. It remains in doubt whether this occurs through inhibition of an oxidative enzyme, or from maintenance of the trapped iodide ion in the reduced state, or through some other mechanism. It is evident, however, that thiourea and related substances do not prevent the *trapping* of iodide by the gland.[1]

The trapped iodide exists in dynamic equilibrium with the iodide ion of the blood. If the trapped iodide is labeled with I^{131} it can be rapidly diluted out by the administration of a relatively large amount of the stable isotope, I^{127}. The administration of thiocyanate ion, an ion sharing many properties in common with iodide, will also result in a discharge of trapped iodide from the gland. Likewise, prior treatment with thiocyanate will prevent the accumulation of I^{131}.

In a systematic survey of anions that might have an effect similar to that of thiocyanate, Wyngaarden, Wright and Ways[2] found that in the rat perchlorate was approximately 10 times as effective as thiocyanate. I^{131} accumulated by the thyroid of the rat receiving propylthiouracil was rapidly discharged when perchlorate was injected, and pretreatment with perchlorate effectively prevented thyroidal accumulation of iodide. The mechanism of action of perchlorate is unknown, but competition for receptor groups of the gland that are responsible for the initial inorganic binding or inhibitory effects on enzyme systems have been suggested.[3, 4]

Perchlorate appears to have had little pharmacological attention. Kahane[4] observed the effects of intravenous injections on four rabbits; 250 mg. of $NaClO_4$ was without effect, but 500 mg. by intracardiac injection caused a transient paralysis of the hind limbs, and in two animals diarrhea. Eichen[5] found the effect of perchlorate on the frog heart to be identical with that of thiocyanate. He administered 1 to 2 gm. orally to patients and observed no ill-effects. Seventy per cent was recovered in the urine in 12 hours and 85% to 90% in 24 hours.

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Durand[4] gave a patient 7S4 mg. of $NaClO_4$ and found it in the urine 15 minutes later. At three hours 30% had appeared in the urine. He found no evidence methemogoblin formation.

The similarity of action of perchlorate on the thyroid of the rat to thiocyanate suggested an extension of these observations to the human The results herein reported indicate that the effects in thyrotoxic patients s similar.

Methods

The subjects of this investigation were patients with Graves' disease who we attending the Thyroid Clinic of the Massachusetts General Hospital. In almost all there had been no recent treatment for the disease, but an occasional patient had been receiving 1-methyl-2-mercaptoimidazole for a variable period of tim The initial observations were always made at least a month after discontinuan of all therapy.

The antithyroid drug employed was 1-methyl-2-mercaptoimidazole, giv orally in a single 30-mg. dose. Tracer doses of I^{131} of approximately 10 micr curies were used. Measurements for the most part were obtained with a lead-shielded scintillation counter employing a sodium iodide crystal.[3] The crystal was approximately 10 cm. from the anterior surface of the neck, and the le shielding was in contact with the neck. The sensitivity of the machine was approximately 4000 counts per microcurie at the same distance. Counting rates from the anterior part of the thigh were obtained at the same time. The obse vations on the first four patients were obtained with a four-quadrant scintill tion counter.[3] The sensitivity of this device was approximately 1000 counts per microcurie at the geometrical center of the array. The findings with the tw devices were in all respects comparable. Forty-eight hour I^{131} accumulations i the glands were obtained from the four-counter array by comparing the patients with a suitable standard and employing a predetermined correction factor. T appreciable radioactivity was present in the neck at the time of a second a larger tracer was given so as to minimize the background correction.

A series of observations was made on 12 patients with typical Graves' disease. The experiments were of three types. In one the subjects received a blockin dose of 1-methyl-2-mercaptoimidazole, then a tracer of I^{131}, and, when this ha accumulated in the gland, an oral dose of $KClO_4$. In the second series the $KClO_4$ was given before the tracer. In the third group the blocking drug was omitted and the $KClO_4$ given prior to the tracer.

Results

Perchlorate Discharge of Accumulated Iodide

To eight patients 30 mg. of 1-methyl-2-mercaptoimidazole was given orally, and to a ninth a dose of 200 mg. of propylthiouracil was administered. Approximately one hour later a tracer of I^{131} was given. The accumulation of this in th neck was recorded at frequent intervals until it was leveling off or slowly d clining. At this point quantities of $KClO_4$ varying from 3 to 500 mg. were given orally in small volumes of water. In each patient, except the propylthiouraci treated patient, there was a sharp fall in the counting rate within a few minut

after ingestion of $KClO_4$. This always occurred within 30 minutes. With smaller doses the discharge of the I^{131} was incomplete, but doses of 100 mg. caused a fall in counting rates nearly to, or in one case slightly below, the counting rates

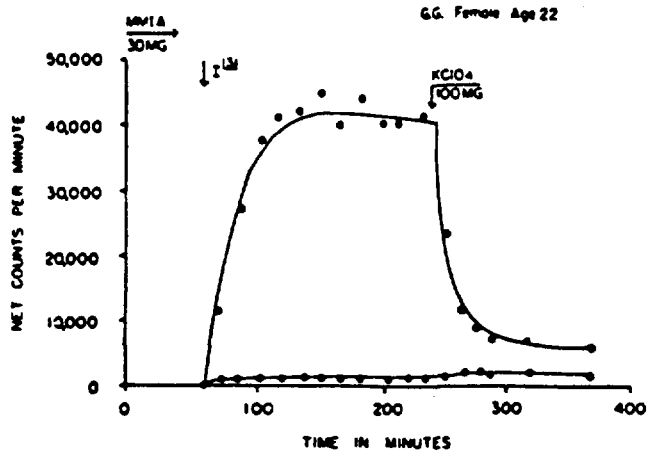

FIG. 1.—Perchlorate discharge of accumulated iodide. At zero time the patient received 30 mg. of 1-methyl-2-mercaptoimidazole. At the first signal, a tracer dose of I^{131} was given and at the second signal 100 mg. of $KClO_4$. The upper curve is counts recorded from the thyroid and the lower those recorded from the thigh. The abscissa is time in minutes; the ordinate is net counts per minute.

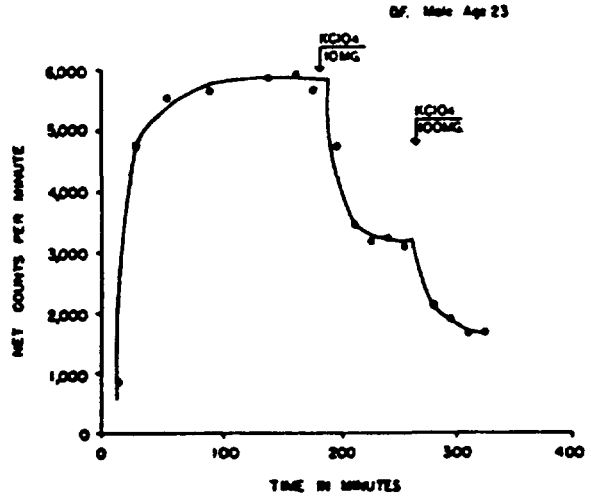

FIG. 2.—Perchlorate discharge of accumulated iodide. Sixty mintues before zero time the patient received 30 mg. of 1-methyl-2-mercaptoimidazole, and at zero time a tracer dose of I^{131}. At the first signal the patient received 10 mg. of $KClO_4$ and at the second signal 100 mg. The abscissa is time in minutes; the ordinate is net counts per minute.

recorded from the thigh. The single instance in which a fall did not occur was in the patient who received propylthiouracil as the blocking agent. The perchlorate was given while the counting rate was increasing. No further increase occurred, and 1 gm. of potassium iodide given orally in solution also failed to cause a fall in counting rate.

Figure 1 illustrates the effect of a single 100-mg. dose of $KClO_4$ on trapped iodide. Only 15% of the initially accumulated I^{131} was present in the neck with a few minutes after administration of the $KClO_4$. At least part of the res labeled iodide recorded from the neck was in the blood circulating through structures of the neck rather than in the gland parenchyma. If as a correction the counting rate recorded over the thigh is subtracted, then it appears that scarcely any I^{131} remained trapped within the thyroid after the perchlorate. In Figure 2 is shown the effect of 10 mg. of $KClO_4$ followed by 100 mg. The

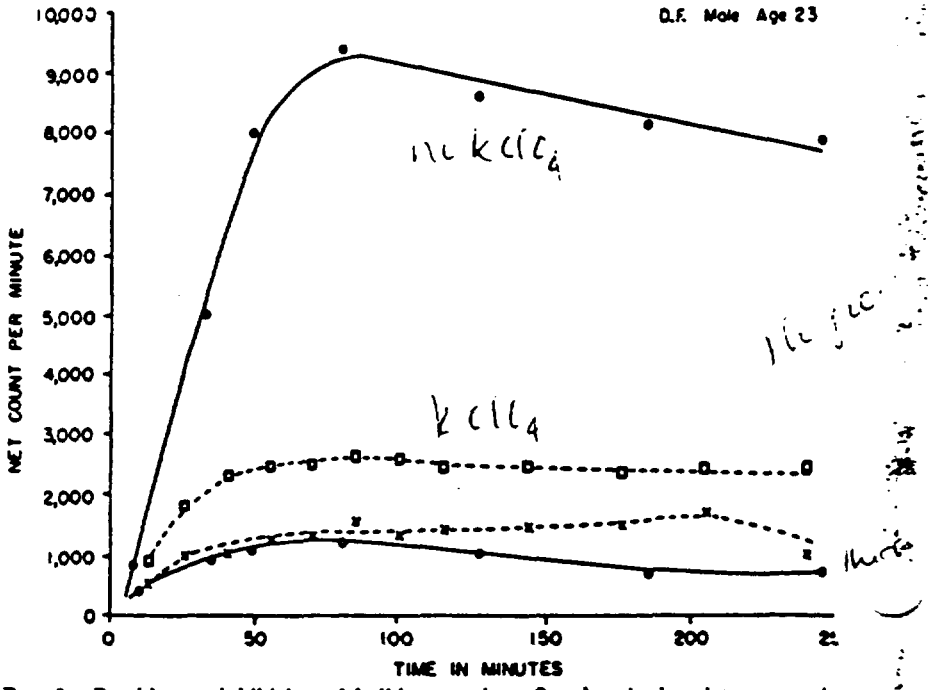

FIG. 3.—Perchlorate inhibition of iodide trapping. One hundred and twenty minutes before zero time the patient received 30 mg. of 1-methyl-2-mercaptoimidazole, and at zero time a tracer dose of I^{131}. The solid curves represent the events when the patient received no $KClO_4$. The dashed curves represent the events when 100 mg. of $KClO_4$ was administered 60 minutes before zero time. In each case the upper curve is counts from the thyroid region and the lower curves counts from the thigh. The abscissa is time in minutes; the ordinate is net counts per minute. The two tracers were adjusted to the same counting rate. Note the inhibition of iodide uptake by the prior administration of $KClO_4$.

smaller dose resulted in an incomplete discharge of the I^{131} and the larger dose in a further discharge. The final counting rate over the gland was approximately four times that from the thigh.

Perchlorate Inhibition of I^{131} Accumulation in the Blocked Gland

Two patients received 30 mg. of 1-methyl-2-mercaptoimidazole, and an hour later 100 mg. of $KClO_4$ orally. One hour later tracers of I^{131} were given. A few days later exactly the same observations were made except that the $KClO_4$ was omitted. $KClO_4$ inhibited the accumulation of I^{131} by the thyroid. This is illus-

trated in Figure 3. The patient was a young man with severe thyrotoxicosis. The accumulation of I^{131} in his thyroid when he was pretreated with perchlorate was only 27% of what it was when no perchlorate was given. At least a portion of the I^{131} must have been in the blood of his large and vascular gland rather than in the parenchyma of the gland. When the correction from counting rates over the thigh is made, this value becomes 13%. In the second patient the difference was even more striking. The counting rates from the neck were the same as those from the thigh after administration of $KClO_4$.

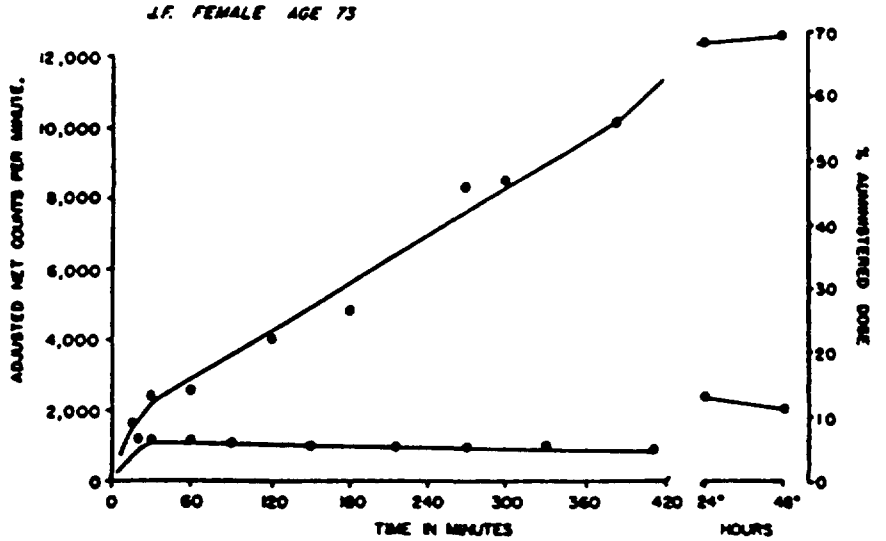

FIG. 4.—Perchlorate inhibition of iodide trapping. The lower curve represents the events when 100 mg. of $KClO_4$ was administered orally one hour prior to zero time. The upper curve was obtained without prior administration of $KClO_4$. The tracers were given at zero time and were adjusted to the same counting rate. The abscissa is time in minutes; the ordinate is counts per minute, or percentage of administered dose in the thyroid.

Perchlorate Inhibition in the Unblocked Gland

Three patients received tracers of I^{131} an hour after being given 100 mg. of $KClO_4$. No 1-methyl-2-mercaptoimidazole was given. Several days later each patient received a control tracer of I^{131} without previous perchlorate. In two, the studies were continued for 48 hours, but in the third a period of observation of only five hours was possible after the tracer.

The single dose of $KClO_4$ strikingly depressed the accumulation rate of the I^{131}, as well as the 24-hour and 48-hour accumulations by the glands. It was apparent in the two longer experiments that after five or six hours there was an inflection in the counting rate curve in an upward direction. This is illustrated in Figure 4. In this patient the 24-hour and 48-hour uptakes of I^{131} were 12.9% and 11.2%, respectively, of the administered dose when they were pretreated with perchlorate, and 68.0% and 69.2% at 24 hours and 48 hours, respectively, when the uptake studies were done without prior administration of perchlorate. In the second patient the control values were 70.3% and 69.9% of the administered tracer dose present in the gland at 24 and 48 hours; when the patient was

pretreated with $KClO_4$, counting rates corresponding to 21.3% and 21.0% were recorded over the thyroid at 24 and 48 hours, respectively.

Discussion

The antithyroid drugs of the thiourea group have provided a means for rating for study the systems of the thyroid involved in iodide trapping on one hand and hormone synthesis and release on the other. The *iodide of the thyroid* exists in exchangeable equilibrium with the *iodide of the blood*, although there is a considerable concentration gradient in favor of the gland. This iodide has been shown to be nonprecipitable and diffusible.[1] When this space is demarcated with I^{131}, the isotope can be readily diluted out by an excess of I^{127}, and also displaced by an excess of thiocyanate. The latter effect is particularly interesting because a concentration gradient of thiocyanate has not been found.[2] One theory proposed[2] is that these ions are in competition for binding sites on the surface of or within the thyroid cell.

Perchlorate appears to be another substance that can displace iodide from its position in the thyroid gland. The data here presented demonstrate that this occurs with considerable efficiency. A dose as small as 3 mg. given orally produced a considerable fall in thyroidal iodide. Not only is trapped iodide discharged from the glands of patients being treated with the antithyroid drug 1-methyl-2-mercaptoimidazole, but also, if perchlorate is given before the tracer of I^{131}, accumulation of the isotope is inhibited. Perchlorate ion appears, therefore, to have an effect on the thyroid qualitatively similar to that of thiocyanate ion. These data, however, provide no clue to the precise nature of the trapping mechanism or the nature of the effects of perchlorate, or thiocyanate. The similarity of the effects of the two drugs may be only superficial.

The duration of the inhibition of iodide uptake after the oral administration of 100 mg. of perchlorate appeared to be about six hours. Beyond six hours accumulation of I^{131} commenced. Durand[6] found that at this time approximat half the administered dose of perchlorate has been excreted in the urine. A l dose should provide a more prolonged period of inhibition.

The demonstration of perchlorate goiter in rats by Wyngaarden, Wright and Ways[5] and their demonstration of several other simple substances that inhibit iodide uptake suggest that a variety of hitherto unsuspected agents may be operative in the induction of sporadic goiter. The data here presented on human subjects suggest that perchlorate may have a role in therapeutics.

Summary

Aqueous potassium perchlorate, when given in oral doses of 3 to 100 mg., results in a rapid release of previously accumulated iodide from the thyroid glands of human thyrotoxic subjects treated with 1-methyl-2-mercaptoimidazole. Perchlorate also effectively inhibits the accumulation of tracer I^{131}. This action is qualitatively similar to that of thiocyanate.

The period of inhibition of uptake of I^{131} after a single dose of 100 mg. of perchlorate is approximately six hours.

No toxic effects of perchlorate were encountered in these patients, who were given no more than three doses for a total of not more than 600 mg. of the drug.

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Long-term use of potassium perchlorate

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Summary

A case of Graves' disease with potassium perchlorate for 22 years without ill effect is described. Thyrotoxicosis recurred 4 weeks after the medication was withdrawn, suggesting that euthyroidism had been maintained by chronic use of the drug. As toxicity of perchlorate is probably dose related, it is suggested that long-term use of low dose perchlorate may be no more hazardous than alternative antithyroid therapy.

Introduction

Potassium perchlorate was extensively used as an antithyroid agent in the late 1950s and early 1960s (Crooks and Wayne, 1960): by competitive inhibition of the trapping of iodide by the thyroid it was effective in reducing thyroid hormone production by the gland, and consequently in relieving symptoms of thyrotoxicosis (Godley and Stanbury, 1954). No evidence has been produced to suggest that it might influence the natural course of thyrotoxicosis. Following reports of toxicity, in particular of bone marrow hypoplasia (Barzilai and Sheinfeld, 1966) it fell into disfavour, and is now used mainly for investigative purposes. The author now reports a case of long-term use of potassium perchlorate.

Case report

A 72-year-old female was referred to the Thyroid Clinic in August 1980 with symptoms of thyrotoxicosis. She had undergone a partial thyroidectomy in another hospital in 1945 for thyrotoxicosis. In 1956, she was diagnosed as suffering from pernicious anaemia, and started on regular vitamin B_{12} therapy. In 1958, her thyrotoxicosis recurred both clinically and biochemically. She was rendered euthyroid with potassium perchlorate, one g/day by mouth for one month, and maintained thereafter on 200 mg/day, with good control of symptoms. She remained clinically euthyroid on this therapy without ill effect until May 1980, when her GP stopped the potassium perchlorate. Four weeks later she developed symptoms of thyrotoxicosis, including weight loss, heat intolerance and excessive sweating, and was referred to the Thyroid Clinic. Apart from pernicious anaemia affecting a maternal aunt, she gave no other history of note.

On examination she was clinically thyrotoxic, with warm moist palms and hyperkinetic movements. She had a tachycardia of 120 beats/min. A small diffuse goitre was palpable, with the left lobe being larger than the right; no bruit was audible. There was no ophthalmopathy. Other examination was unremarkable.

Initial thyroid function tests confirmed the clinical impression with a T_4 of 245 nmol/l (normal range 59–174), T_3 of 4·2 nmol/l (normal range 1·29–3·3) and a free thyroxine index of 77·4 (normal range 17·8–46·1) (Amersham radioimmunoassay kit). Thyroidal uptake of ^{123}Iat 20 min after i.v. administration of the tracer was elevated at 9·7% of dose (normal range 2–8%); the precipitin test for thyroglobulin antibody was negative. A technetium scan of the thyroid showed a diffuse uptake of isotope, with the left lobe more active than the right. Haemoglobin was 11·3 g/dl with an MCV of 88 fl; WBC was $5·3 \times 10^9$/l, with normal film appearances; platelet count was normal at 194×10^9/l.

A diagnosis of Graves' disease seemed reasonable in view of her history of pernicious anaemia, and the diffuse thyroid scan appearance. In view of her age, and recurrent nature of her illness, she was treated with radioactive iodine (^{131}I) by mouth, and is now clinically euthyroid.

Comment

This appears to be a unique case with maintenance of euthyroidism by the use of potassium perchlorate over a period of 22 years. The temporal relationship between withdrawal of perchlorate and recurrence of thyrotoxicosis suggests that perchlorate was responsible for the maintenance of euthyroidism, by continued chronic intrathyroidal iodine depletion. With the withdrawal of perchlorate, unblocked excessive iodide trapping was able to occur, leading to excessive thyroid hormone production; this

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situation is analogous to the cases of thyrotoxicosis 'unmasked' in populations by the introduction of dietary iodine supplementation (Connolly, Vidor and Stewart, 1970).

This patient received potassium perchlorate for 22 years without any untoward effects. The reports of adverse reactions to potassium perchlorate suggested that these effects of the drug were dose related (Morgans and Trotter, 1960), and it may be that low dose perchlorate (200 mg) is no more toxic than the current generation of thiourylene antithyroid drugs (Barzilai and Sheinfeld, 1966).

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EFFECTS OF METHYLMERCAPTOIMIDAZOLE (MMI), PROPYLTHIOURACIL (PTU), POTASSIUM PERCHLORATE ($KClO_4$) AND POTASSIUM IODIDE (KI) ON THE SERUM CONCENTRATIONS OF THYROTROPHIN (TSH) AND THYROID HORMONES IN THE RAT

By

P. T. Männistö[1], T. Ranta[2] and J. Leppäluoto[3]

ABSTRACT

Male Sprague-Dawley rats were given graded doses of methylmercaptoimidazole (MMI), propylthiouracil (PTU), $KClO_4$ or KI in drinking water for 4 days, or the lowest effective dose of each drug for various times. The rats were sacrificed at 1–2 p.m. and serum T_3, T_4 and TSH concentrations were measured by radioimmunoassays. It was found that administration of 3 mg/l of MMI, 10 mg/l of PTU and 100 mg/l of $KClO_4$ for 4–14 days induced a transient rise in serum TSH and a fall in serum T_3 or T_4 or in both. The effects of KI were not consistent. In another series of experiments, PTU (10 mg/l) was given in drinking water for 4 days, and then graded doses of T_3 or T_4 were given iv. or 100 ng of TRH was injected into a tail vein, or the animals were exposed to 4°C for 30 min. The initial high TSH levels were further increased by TRH and cold and decreased by T_3 and T_4. The PTU-treated animals had goitres after 4 days. We infer that low doses, that is to say 10–100 times lower than previously described, of antithyroid drugs induce a hypothyroidism characterized by an increased TSH level and a decreased serum T_3 or T_4 level or both. A 4 days' treatment with PTU (10 mg/l in tap water) is a suitable tool for studying the effect of various conditions on TSH secretion.

The effects of various antithyroid drugs are well documented at the level the thyroid gland (*Stanley & Astwood* 1947; *Richards & Ingbar* 1959; *Iino et al.* 1961; *Hershman & Van Middlesworth* 1962; *Nagataki & Ingbar* 1964; *Wolff* 1964). However their effects on the serum concentrations of immunoassay T_3, T_4 and TSH have been less widely studied. In most of the previous only one, and evidently a high concentration of an antithyroid drug used, resulting in at least temporarily increased secretion of TSH (*Lawrence* 1964; *Wilber & Utiger* 1967; *Liewendahl et al.* 1972; *Griessen & Lemarchand-Béraud* 1973; *Suberi et al.* 1975).

The effects of suprapituitary factors on the regulation of TSH are usually studied in situations where the secretion of TSH is stimulated. We have previously stimulated TSH secretion in rats by cold-exposure (*Leppäluoto et al.* 1974; *Tuomisto et al.* 1975; *Ranta et al.* 1977) and found that it is possible to modify the stimulated TSH secretion by various drugs influencing central neuro-transmission (*Tuomisto et al.* 1975; *Ranta et al.* 1977; *Männistö et al.* 1979). In other studies TSH secretion was stimulated by thyroidectomy but very high TSH levels were not changed by drugs (*Mueller et al.* 1976) or cold-exposure (*Männistö*, unpubl. results).

To find another reliable system for the stimulation of TSH secretion and elucidate the acute effects of various antithyroid drugs, we measured serum concentrations of T_3, T_4 and TSH by specific radioimmunoassays in a series of experiments where graded doses of four antithyroid drugs were given to rats in tap water for 4 days. In the further studies a low effective dose of each drug was given for varied periods of time. We were able to show that very low concentrations of antithyroid drugs effectively stimulated TSH secretion. The elevated TSH levels responded to exposure to cold, to thyroid hormones and to TSH-releasing hormone.

MATERIAL AND METHODS

Animals

Male outbred Sprague-Dawley rats weighing 150–220 g at the beginning of the experiments were used. They were kept 2–3 animals per cage and fed tap water and pellets (iodine concentration 0.5–1 mg/kg) *ad libitum*. The animal room was artificially illuminated from 7 a.m. to 7 p.m. and kept at 20–22°C. The animals were decapitated between 1 p.m. and 3 p.m.

Experimental designs

1. ***Studies with various doses of propylthiouracil (PTU), methylmercaptoimidazole (MMI), $KClO_4$ and KI.*** – In the *first* series of experiments graded doses of PTU (0, 1, 3, 10, 25 and 50 mg/l), MMI (0, 1, 3, 10 and 25 mg/l), $KClO_4$ (0, 10, 50, 100, 300 mg/l) and KI (0, 10, 100 and 1000 mg/l) in tap water were given to groups of 5

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rats for 4 days beginning at 1 p.m. on the first day. The consumption of drinking water was similar in all the groups. The daily dose of each drug at 10 mg/l level varied mg. All animals gained weight similarly and were apparently healthy. The animals were decapitated on the 5th day at 1–3 p.m. and the whole trunk blood was collected for the measurement of serum T_3, T_4 and TSH concentrations (cf. below).

In the second series of experiments PTU (10 mg/l), MMI (5 mg/l), $KClO_4$ (100 mg/l), and KI (100 mg/l) were given in tap water as above and the animals (n = 5–7 in each group) were decapitated on the 2nd, 4th, 6th, 9th and 14th day at 1–3 p.m. One or two control rats were killed at each point of time (total 6–9 controls per each drug).

In the third experiment PTU (10 mg/l) was given in drinking water as above and on the 4th (n = 9) and 10th day (n = 6) the animals were decapitated. The control rats (n = 10) were killed on the 10th day. The thyroid glands and adenohypophyses were excised and weighed.

2. Effects of cold, TRH and thyroid hormone treatment on TSH concentration in the 4-day PTU treated rats. – In the *first* series of experiments the reproducibility of the PTU-induced TSH response was studied. PTU (10 mg/l) was given in drinking water for 3 or 4 days and then the animals were decapitated for the measurement of serum TSH. The experiments (5–7 rats in each) were repeated 5 times and the coefficients of variation in the final serum TSH concentrations were calculated.

In the *second* experiment PTU (10 mg/l) was given in drinking water as above. On the 4th day 10 rats were transferred to a room with a temperature of 4°C for 30 min and then sacrificed for measurement of serum TSH. Other 10 rats were given 100 ng of TRH into a tail vein and the animals were decapitated 10 min later. The control animals (n = 10) received saline iv and were decapitated at the same time. The fourth group of rats (n = 10) received water instead of PTU.

In the *third* experiment 60 rats received PTU (10 mg/l) as above for 4 days. Then graded doses of T_3 (0, 1.25, 2.5, 5, 12.5 or 125 μg/kg) or T_4 (5, 25, 37.5, 50, 100 or 175 μg/kg) were injected into the tail vein. The rats were decapitated 2 h later for measurement of serum TSH. This time interval has been found suitable in an earlier study in rats (*Wilber & Utiger* 1967).

Hormones and drugs

Thyroxine, triiodothyronine, propylthiouracil, methylmercaptoimidazole, $KClO_4$ and KI were purchased from Sigma (St. Louis). TSH-releasing hormone was obtained from Calbiochem (San Diego).

Radioimmunoassays of serum T_3, T_4 and TSH

T_3 and T_4 antisera were purchased from Farmos (Turku). T_3 antiserum had a cross-reactivity of < 0.01 % against mono- or diiodotyrosine and 0.15 % against T_4. T_4 antiserum had a cross-reactivity of < 0.01 % against iodotyrosines and 0.55 % against T_3. ^{125}I-labelled T_3 and T_4 were purchased from Amersham, England. A 100 μl serum sample was incubated overnight with antiserum and tracer at 4°C in a buffer containing anilinonaphtalen-sulphonate. The immunocomplex was precipitated with polyethyleneglycol (final concentration 12.5 % w/v). Serum TSH was measured with a rat TSH kit obtained as a gift from NIAMDD. A rat TSH preparation RP-1 was used as a standard (*Ranta* 1975).

RESULTS

Effects of graded doses of antithyroid drugs administered for 4 days, on serum T_3, T_4 and TSH concentrations

PTU. – At the PTU level of 1 mg/l there were no significant ch in serum hormone levels in 4 days. At 5 mg/l serum TSH rose from 58 ng/ml ($P < 0.05$) and remained at about that level at higher doses. Stati cally significant falls in serum T_3 and T_4 occurred at 10 mg/l or at higher doses (Fig. 1).

MMI. – MMI did not affect serum hormone concentrations at 1 mg/l c level in 4 days. At 5 mg/l serum TSH increased from 640 to 2150 ng/ml ($P < 0.01$) whereas serum T_3 and T_4 were not significantly affected. At and 25 mg/l level of MMI, serum TSH was still high but declining. Serum concentrations decreased from 52 to 37 nmol/l at 10 mg/l ($P < 0.05$) and to 32 nmol/l at 25 mg/l ($P < 0.01$). The serum T_3 concentrations remained changed at all MMI dose levels (Fig. 2).

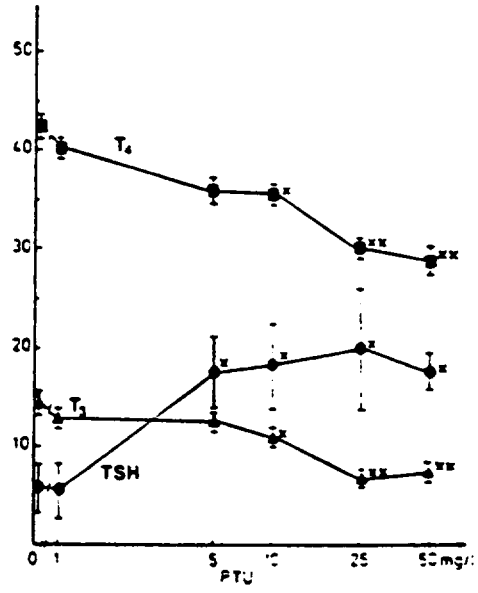

Fig. 1.

Serum T_3 (▲—▲: 10^{-1} nmol/l), T_4 (■—■: nmol/l) and TSH (●—●: 10^{2} ng/ml) concentrations as a function of the PTU dose (mg/l in drinking water; log scale) in the rat. Mean ± SEM. n = 5–6. Statistics: x $P < 0.05$, xx $P < 0.01$ vs. the water controls

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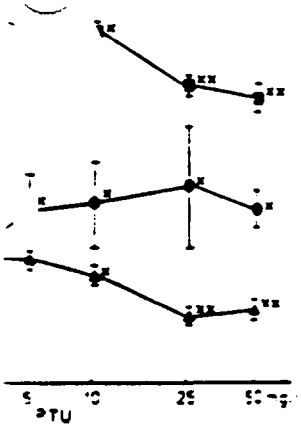

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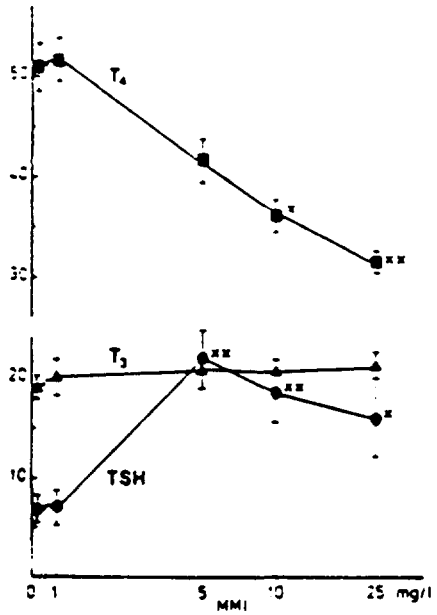

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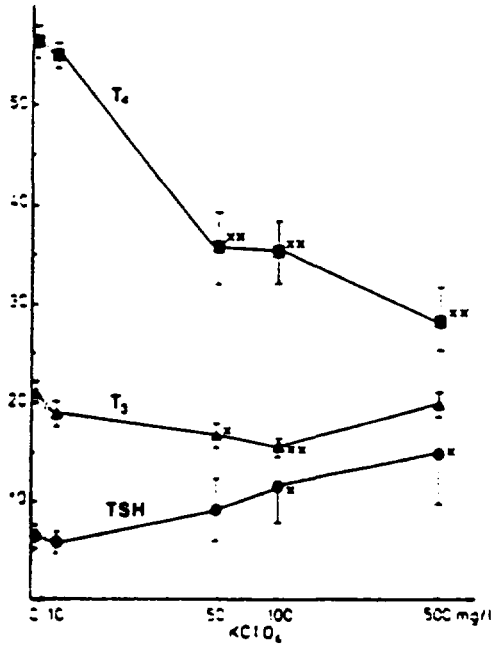

Fig. 3.
...erum T_3, T_4 and TSH concentrations as a function of the $KClO_4$ dose (mg l in drinking water: log scale). For further information, see Fig. 1.

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Table 1.

Serum T_4 (nmol/l), T_3 (nmol/l) and TSH (ng/ml) concentrations as a function of ti during PTU (10 mg/l), MMI (5 mg/l), $KClO_4$ (100 mg/l), and KI (100 mg/l) treatme The drugs were given in tap water.

	Duration of the treatment, days					
	0	2	4	6	9	
T_4, nmol/l						
PTU	50.0 ± 4.0	54.8 ± 5.5	58.0 ± 7.0*	27.4 ± 4.1**	12.1 ± 1.6**	34.8 ± 9
MMI	50.8 ± 3.1	49.0 ± 3.2	45.4 ± 2.8	36.8 ± 4.1*	–	34.7 ± 2
$KClO_4$	52.1 ± 6.5	40.8 ± 2.6	47.5 ± 2.7	45.4 ± 2.5	–	–
KI	69.4 ± 3.0	45.1 ± 3.2	63.5 ± 2.9	74.5 ± 0.9*	61.8 ± 4.3	52.7 ± 2
T_3, nmol/l						
PTU	1.8 ± 0.3	0.5 ± 0.07**	0.7 ± 0.14*	0.6 ± 0.05**	0.6 ± 0.13**	0.8 ± 0.1
MMI	2.0 ± 0.12	2.4 ± 0.12	2.1 ± 0.21	1.9 ± 0.15	–	2.3 ± 2
$KClO_4$	2.0 ± 0.10	2.1 ± 0.16	1.6 ± 0.11	1.6 ± 0.06	–	–
KI	2.8 ± 0.22	1.8 ± 0.07*	2.5 ± 0.16	2.9 ± 0.10	2.2 ± 0.07	2.7 ± 0.1
TSH, ng/ml						
PTU	520 ± 60	980 ± 100*	1400 ± 75*	2450 ± 350**	4550 ± 410**	1500 ± 50
MMI	505 ± 130	750 ± 20	2300 ± 250**	990 ± 170*	–	500 ± 250
$KClO_4$	610 ± 110	720 ± 160	1120 ± 100*	1095 ± 140	–	1200 ± 1
KI	650 ± 50	1460 ± 240*	1340 ± 250	1090 ± 230	1350 ± 300	570 ±

Mean ± SEM. n = 5–8. Statistically significant changes from the control values (= 0 day are shown as follows: * $P < 0.05$, ** $P < 0.01$.

$KClO_4$. – At 10 mg/l dose level serum hormones were unchang a 50 mg/l T_3 fell from 2.1 to 1.7 nmol/l ($P < 0.05$) and serum T_4 from 56 t 36 nmol/l ($P < 0.01$). Similarly, T_4 remained low at 100 and 500 mg/l is level and T_3 at 100 mg/l dose level. Serum TSH rose at 100 mg/l from 1 to 1170 ng/ml ($P < 0.05$) and continued to rise to 1490 ng/ml at 500 mg ($P < 0.01$) (Fig. 3).

KI. – There were no statistically significant changes in the hormone concen trations at 10–1000 mg/l dose levels of KI in 4 days (data not shown).

Serum T_3, T_4 and TSH concentrations during administration of the four antithyroid drugs for various times

PTU. – When PTU (10 mg/l) was given, serum T_3 fell on the 2nd day f m 1.8 to 0.5 ng/ml ($P < 0.01$) and remained at this low level for several d s

Table 1.

TSH (ng/ml) concentrations as a function of $KClO_4$ (100 mg/l) and KI (100 mg/l) treatment. Drugs were given in tap water

	ion of the treatment, days			
4	6	9		
38.0 ± 7.0*	27.4 ± 4.1**	12.1 ± 1.6**	34.8 ±	
4 ± 2.5	36.5 ± 4.1*	–	34.7 ±	
.3 ± 2.7	45.4 ± 2.5	–		
63.5 ± 2.9	74.8 ± 0.9*	61.5 ± 4.3	82.7 ±	
0.7 ± 0.14*	0.6 ± 0.05**	0.6 ± 0.15**	0.8 ±	
2.1 ± 0.21	1.9 ± 0.15	–	2.3 ±	
6 ± 0.11	1.6 ± 0.06	–		
3 ± 0.16	2.9 ± 0.10	2.2 ± 0.07	2.7 ±	
0 ± 75*	2450 ± 350**	4550 ± 410**	1500 ±	
0 ± 250**	990 ± 170*	–	800 ±	
120 ± 100*	1095 ± 140	–	1200 ±	
340 ± 250	1090 ± 230	1350 ± 300	570 ±	

nificant changes from the control values (* $P < 0.05$, ** $P < 0.01$.

l serum hormones were unchanged, but ...nol/l ($P < 0.05$) and serum T_4 from 5... T_4 remained low at 100 and 500 mg/l ... el. Serum TSH rose at 100 mg/l from ... itinued to rise to 1490 ng/ml at 500 mg/l

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...was given, serum T_3 fell on the 2nd day from ... mained at this low level for several days.

Serum T_4 fell from 50 to 38 nmol/l ($P < 0.05$) on the 4th day and reached a very low minimum (12.1 nmol/l, $P < 0.01$) on the 9th day. Serum TSH rose from 320 to 2450 ng/l on the 6th day ($P < 0.01$) and increased further to a peak maximum of 4550 ng/ml on the 9th day ($P < 0.01$), and then fell to 1500 ng/ml on the 14th day (Table 1).

The weights of anterior pituitaries were increased and the rats had goitres as early as on the 4th day of the PTU-treatment (Table 2).

MMI. – With 5 mg/l of MMI in drinking water, serum TSH rose from 505 to 2000 ng/ml on the 6th day ($P < 0.01$), then fell to 990 and 800 ng/ml on the 9th and 14th day, respectively. The fall in serum T_4 was significant on the 6th ($P < 0.05$) and 14th day ($P < 0.01$). Serum T_3 remained unchanged during the experiment (Table 1).

$KClO_4$. – When 100 mg/l of $KClO_4$ was given, serum TSH rose from 610 to 1120 ng/ml on the 4th day remained at that level. Serum T_3 decreased from 2.0 to 1.6 mmol/l on the 4th and 6th day. Serum T_4 also decreased from the 2nd day but the change was not statistically significant in this experiment (Table 1).

KI. – When KI (100 mg/l) was administered in drinking water, serum TSH rose from 650 to 1460 ng/ml on the 2nd day ($P < 0.05$) but then declined to the initial level. Serum T_4 tended to fall at the beginning of the treatment but increased on the 6th day from 52 to 74.8 nmol/l ($P < 0.05$) and on the 14th day to 82.7 nmol/l ($P < 0.01$). Serum T_3 concentration was decreased on the 2nd day only (Table 1).

Table 2.

The weights of the thyroid and anterior pituitary glands of the rats during administration of PTU (10 mg/l) in drinking water for 4 or 10 days.

	Wet weight (mg/100 g of body weight)	
	Thyroid gland	Anterior pituitary
Control	5.8 ± 0.3	2.6 ± 0.1
4 days on PTU	13.3 ± 0.1**	3.5 ± 0.1*
10 days on PTU	16.0 ± 0.2**	3.4 ± 0.1*

Mean ± SEM of 6 animals in each group. * $P < 0.05$, ** $P < 0.01$ vs. the control rats.

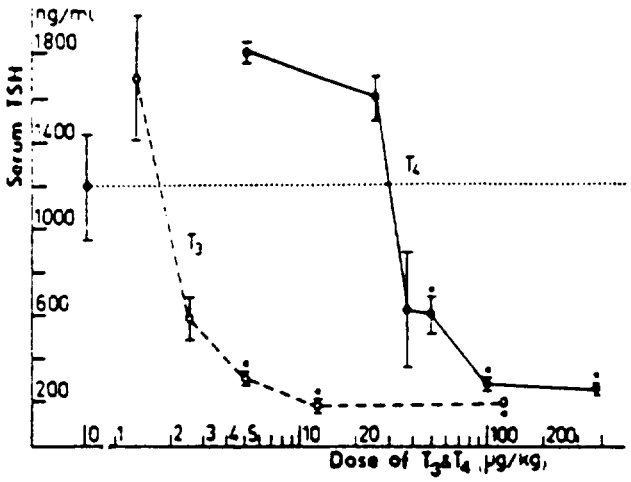

Fig. 4.

Effect of graded doses of T_3 (o- - - -o: 0–125 μg/kg iv; log scale) and T_4 (●— 5–375 μg kg iv; log scale) on the serum TSH levels elevated by a prior PTU treat (10 mg l in drinking water for 4 days). The rats were killed 2 h after the injection o saline. T_3 or T_4. n = 4–5 at each dose level. Mean ± SEM. Statistics: ★ $P < 0.0^5$ vs the PTU control rats (. . . .).

Effects of various manipulations on serum TSH concentration in the PTU-treated animals

Reproducibility. – When PTU (10 mg/l) was given to groups of 5–7 rats 5 separate experiments. serum TSH rose from 441 ± 52 to 906 ± 104 ng (mean ± SEM) in 3 days and from 600 ± 51 to 1720 ± 125 ng/ml in 4 d The respective coefficients of variation in TSH concentrations were 30 % (3 days' treatment) and 16 % (4 days' treatment).

Table 3.
The effect of cold exposure and TRH on the serum TSH levels in the PTU-treated rats (10 mg/l in drinking water for 4 days).

Treatment	Serum TSH (ng/ml)
Water control	446 ± 51
PTU control, 22°C	1173 ± 169
PTU and 30 min at +4°C	2177 ± 277*
PTU and 100 ng of TRH iv	2161 ± 150*

Mean ± SEM of 9–10 animals.
* $P < 0.01$ vs. the PTU control.

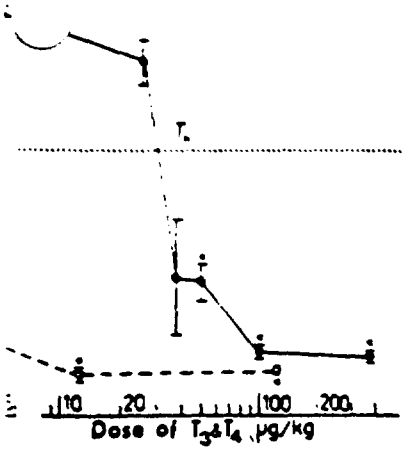

Fig. 4.
- : 0–125 µg kg iv: log scale and T_4 (●
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e rats (10 mg l in drinking water
4 days).

	Serum TSH (ng ml)
	446 ± 91
	1173 ± 169
C	2177 ± 277*
H iv	2161 ± 150*

rol.

Effect of thyroid hormones, TRH and cold-exposure. – In the first experiment the rats were given PTU (10 mg l, 4 days) and then various amounts of T_3 or T_4 iv. Within 2 h small amounts of T_3 (1.7 µg kg) and T_4 (5–30 µg kg) did not significantly modify TSH levels but higher doses rapidly decreased the serum TSH levels (Fig. 4). In another experiment the high TSH levels, induced by PTU, were further increased by iv injection of 100 ng of TRH (from 1173 ± 169 to 2177 ± 277 ng ml, $P < 0.01$) and by transferring the rats from 22 to 4 C for 30 min (to 2210 ± 80 ng ml, $P < 0.01$) (Table 3).

DISCUSSION

In this study each antithyroid drug induced a different pattern of serum immunoassayable hormone levels at the beginning of treatment. We observed that in the PTU-treated rats T_3 fell and TSH increased early and T_4 decreased later. In the $KClO_4$-treated rats T_3 and T_4 decreased at a parallel rate and TSH levels increased at the same time. Administration of MMI did not affect T at all, and after KI serum T_4 was even increased, although serum TSH levels were at least transiently increased in both cases.

Although in this study serum thyroid hormone levels did not fall *before* the rise in serum TSH, we still believe that these antithyroid drugs primarily decrease either serum T_3 or T_4 or both, which, according to the classical feedback theory, then leads to a rise in serum TSH. We want to point out that in the rats kept on a low iodide diet serum TSH was also increased *before* any detectable change in serum T_3 or T_4 (*Riesco et al.* 1977). The fact that we were not able to observe in all cases significant falls in serum thyroid hormone levels before the rise of serum TSH may be due to the inability of T_3 and T_4 radioimmunoassays to detect minute, but possibly physiologically significant, T_3 and T_4 changes.

In earlier studies antithyroid drugs have been used in drinking water in concentrations of 100–1000 mg l (*Bakke & Lawrence* 1964; *Wilber & Utiger* 1967; *Lerwundahl et al.* 1972; *Griessen & Lemarchand-Béraud* 1973), and those treatments have increased serum TSH levels in 1 day – 4 months. Our results show that these doses are unnecessarily high since significant changes in serum T_3, T_4 and TSH were obtained at doses 10–200 times lower. The disappearance of the initial serum TSH rise in response to PTU (*Griessen & Lemarchand-Béraud* 1973) or thyroidectomy (*Van Rees* 1966) is said to be due to the exhaustion of pituitary TSH reserves. We were able to confirm the transient rise in serum TSH levels even with very low doses of PTU as well as with MMI, and to some extent with KI. On the other hand, $KClO_4$ seemed to be able to stimulate TSH secretion continuously. Possibly the dose was relatively lower than that of PTU and MMI, and did not cause the depletion of pituitary TSH.

It was also demonstrated here that MMI did not affect serum T_3 level, which was clearly decreased by PTU. This result was not unexpected because substantial amounts of serum T_3 are derived from serum T_4 by deiodination. PTU – but not MMI – is known to block this reaction (*Van Arsdel & [illegible]* 1956; *Hershman & Van Middlesworth* 1962; *Morreale de Escoba[illegible] del Rey* 1967). We also found that our initial TSH burst was associated with the fall of either serum T_3 (PTU and $KClO_4$) or T_4 (MMI). It is difficult to say whether T_3 or T_4 is able to inhibit TSH secretion at the anterior pituitary level because there are pituitary receptors for both hormones (*Oppenheimer et al.* 1976) but, on the other hand, T_4 is rapidly deiodinated to T_3 in the anterior pituitary (*Silva et al.* 1978).

In this study KI administration also led to the initial TSH burst. Later serum T_4 level began to rise and serum TSH level decline. So it appears that low KI doses (about 4 mg/day) initially work antithyroidally but later, perhaps due to increased availability of iodide, T_4 synthesis is increased. There is no previous information about the effects of small doses of iodide on serum TSH but high doses have slightly increased serum TSH level at 4 months (*Liewendahl et al.* 1972).

The present results prompted us to set up a model in which stimulated TSH secretion can be studied. PTU is given in drinking water (10 mg/l) for 4 days to increase serum TSH levels. The reproducibility of this TSH response is good, even better than that obtained with the cold-exposed animals (*Tuomisto et al.* 1975; *Männistö et al.* 1978). Our model appears to be useful because elevated TSH levels are further increased in response to cold or to administration of TRH and rapidly decreased by thyroid hormones. Both the TRH-induced and cold-induced TSH responses are well comparable with those observed in the normal rats (*Tuomisto et al.* 1975; *Ranta et al.* 1977; *Männistö et al.* 1979). We have also shown that various drugs can modify the PTU-induced TSH secretion (*Männistö & Ranta* 1978).

ACKNOWLEDGMENTS

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: that MMI did not affect serum T_3 level.
This result was not unexpected because
e derived from serum T_4 by deiodination
n to block this reaction (*Van Arsdel & Wil*
sworth 1962; *Morreale de Escobar & Es*
r initial TSH burst was associated
$KClO_4$ or T_4 MMI). It is difficu
inhibit TSH secretion at the anterior pituit
v receptors for both hormones (*Oppenhe*
and. T_4 is rapidly deiodinated to T_3 in the

also led to the initial TSH burst. Later
n TSH level decline. So it appears that lo
work antithyroidally but later, perhaps
T_4 synthesis is increased. There is no pre
small doses of iodide on serum TSH but
-um TSH level at 4 months (*Liewendahl*

us to set up a model in which stimulated
given in drinking water (10 mg/l) for 4
reproducibility of this TSH response is
with the cold-exposed animals (*Tuomisto*
model appears to be useful because elev
in response to cold or to administratio
.hyroid hormones. Both the TRH-induced
well comparable with those observed in
5; *Ranta et al.* 1977; *Männistö et al.*
various drugs can modify the PTU-ind

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RESULTS OF A FOURTEEN DAY ORAL-DOSING TOXICITY STUDY OF AMMONIUM PERCHLORATE

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ABSTRACT

The use of ammonium perchlorate (AP), CAS registry number 7790-98-9, in the manufacture of solid rocket motors has resulted in soil and water contamination. Remediation levels proposed by the US EPA are extremely conservative because of an insufficient toxicity database (ECAO, 1992). Since the thyroid is the target organ and perchlorate inhibits iodine uptake by the thyroid, this 14-day study evaluated the potential of AP to produce changes in levels of triiodothyronine (T3), reverse T3 (rT3), T4, thyroid stimulating hormone (TSH), and thyroglobulin (Tg) by providing specific dose-response information for use in selecting doses for use in a 90-day subchronic toxicity study.

INTRODUCTION

This study was conducted to evaluate the potential of AP to produce alterations in thyroid function and to estimate the threshold dose for AP caused effects on thyroid hormone levels in rats. Results of this pilot study will enable selection of appropriate doses for a subchronic study that will evaluate the potential of ammonium perchlorate (AP) to produce alterations in paternal fertility, maternal pregnancy and lactation, growth and development of offspring of Sprague-Dawley rats. The animals received the test compound in drinking water as this route of treatment provides a more uniform dose than a single bolus dose produced by gavage and simulates the most probable route of human exposure in cases of environmental contamination. Blood levels of Tg, T_3, T_4, rT_3, and TSH were determined.

MECHANISM OF TOXICITY

Many goitrogenic xenobiotics that increase the incidence of thyroid tumors in rodents exert a direct effect on the thyroid gland to disrupt one of several possible steps in the biosynthesis and secretion of thyroid hormones. Perchlorate is known to competitively inhibit one of these steps, the iodine trapping mechanism, which incorporates free iodine into T_3 and T_4 (Capen, 1992). Low T_3 causes release of thyroid stimulating hormone (TSH) from the anterior pituitary, which results in thyroid follicular cell stimulation and hyperplasia. This hyperplasia may cause an increase in thyroid gland size (hypertrophy) (ORD, 1988).

The primary objective of this range-finder study was to determine toxicity information to establish doses of AP for a 90-day subchronic toxicity study. Sprague Dawley rats were exposed to varying concentrations of AP in their drinking water for a two-week period. The rats were then sacrificed and their thyroid hormone levels were measured by a radioimmunoassay technique. An additional goal of this work was to estimate a threshold dose based on changes from the control thyroid hormone data.

Data obtained from this study will be used to statistically estimate the threshold level for AP effects on the thyroid, the target organ for toxicity. The threshold level will be considered to be the "lowest observed adverse effect level" (LOAEL). The next lowest dose will be the "no observed adverse effect level" (NOAEL) and will be used for determination of the RfD for ammonium perchlorate using standard USEPA methodology. The dose-response data, reproductive and developmental toxicity information, and effects of AP on other organs, coupled with the establishment of a threshold dose for thyroid hormone effects will remove much of the uncertainty surrounding the provisional RfD and permit calculation of a RfD that should be at least an order of magnitude higher. Doses for the 90-day subchronic study will be based on results from this pilot study.

RELEVANCE OF RESEARCH

We wish to emphasize the need for this research by describing the lack of toxicity information, and specifically the lack of dose-response data for AP. Furthermore, it is intended to familiarize the reader with the mechanism by which thyroid hormone levels are inhibited by AP. Hormones are products of living cells that circulate in body fluids and produce a specific effect on the activity of cells remote from their point of origin. This inability of the thyroid gland to produce thyroid hormones, if not corrected, leads to excessive development of thyroid tissue cells (hypertrophy), an unusual increase in the number of tissue cells (hyperplasia) and the formation of abnormal masses of tissue (tumors) that possess no physiologic function (neoplasia) in experimental animals as well as in humans (Capen, 1992; Hill et al., 1989; Paynter et al., 1988).

The Thyroid Gland

This section explains the nature, formation, and secretion of the thyroid hormones and discusses the mechanisms by which circulating levels of the hormones are regulated. Thyroxine (T4) and triiodothyronine (T3) are classically regarded as the two hormones produced by the thyroid gland. They contain 4 and 3 atoms of iodine, respectively, and are abbreviated as T4 and T3 according to their iodine content. These hormones are synthesized in the thyroid gland by iodinating thyroglobulin (Tg), an iodine containing protein stored in the thyroid (Goodman and van Middlesworth, 1980). This process can be divided into four steps. The first stage in the synthesis of thyroid hormones is the uptake of iodide from the blood by the thyroid gland. The trapped iodide is then combined with oxygen (oxidized). Once it is oxidized, iodine rapidly iodinates tyrosine residues within the Tg molecule to form monoiodotyrosine (MIT) and diiodotyrosine (DIT) in a process is called iodide organification. Finally, either two DIT molecules or one DIT and one MIT molecule combine to form T4 and T3 respectively, in the ratio of 5:1, so that most of the hormone released is T4.

Under normal conditions the thyroid may concentrate iodide up to 25 times higher than the blood concentration. This ratio may be considerably higher (250:1) when the thyroid is active. Iodide uptake may be blocked by several anions, one of which is perchlorate (Goodman and van Middlesworth, 1980).

T4 is the major hormone secreted from the thyroid and is converted to more active T3 in a variety of peripheral tissues, including the pituitary gland. T4 is also metabolized to rT3 which is hormonally inactive and has no know function, except perhaps as an inhibitor of the conversion of T4 to T3 (Hill et al., 1989; Stevens, 1985, Goodman and van Middlesworth, 1980).

Homeostatic control of thyroid hormone synthesis and secretion in the thyroid gland is effected by a sensitive feedback mechanism that responds to changes in circulating levels of the thyroid hormones T4 and T3. The mechanism involves the anterior pituitary of the brain (Hill et al., 1989; Paynter et al., 1988; Houk, 1980). Thyroid-stimulating hormone (TSH, or thyrotropin), which is secreted by the anterior pituitary gland and causes the thyroid to create new thyroid hormones, is very important in the feedback mechanism. It independently promotes iodide trapping and iodination of Tg. The rate of release of TSH from the pituitary is controlled by the circulating levels of T4 and T3.

If for any reason there is a decrease in circulating levels of thyroid hormones, TSH is secreted and thyroid function is increased. If exogenous thyroid hormone is administered, eventually the thyroid gland becomes inactive and atrophied. The blood concentrations of both T4 and T3 are important factors in the release of TSH (Capen, 1992; Hill et al., 1989; Paynter et al., 1988; Goodman and van Middlesworth, 1980).

According to Goodman and van Middlesworth (1980), an exact description of the role of the thyroid hormones is not yet possible. However, they discuss several studies which indicate that if the hormones are not present in the early stages of life, mental maturation, bone development, and the central nervous system are negatively affected. In some instances, the lack of bone development can be corrected by administering T4. However, administration of even tremendous amount of T4 does nothing to correct

mental retardation, suggesting that the hormones must be present during critical periods in order for normal development to occur.

Thyroid Gland Neoplasia

Hill et al explains that thyroid neoplasia may be induced by exposure of experimental animals to a variety of treatment regiments, chemicals produced outside the body (exogenous), or physical agents. "It has been recognized for some time that neoplasms induced in experimental animals by a number of these treatments result from thyroid gland dysfunction, in particular, [enlargement of the thyroid gland and increased metabolic rate] hypothyroidism." Factors inducing hypothyroidism include iodine deficiency, surgically removing part of the thyroid gland, and the transplantation of TSH-secreting pituitary tumors. "The one factor common to each of these conditions is that they all lead to increased production of TSH and prolonged stimulation of the thyroid gland by "excess" TSH." Whatever the cause (i.e. low iodine diet, blocked iodide uptake by an anion), prolonged stimulation of the thyroid-pituitary feedback mechanism that results in the release of elevated levels of TSH by the pituitary may lead to thyroid gland neoplasia. However, thyroid hyperplasia and neoplasia in these cases can be blocked by doses of exogenous thyroid hormone or by surgically removing the pituitary gland (hypophysectomy) (Hill et al., 1989).

A recent review of chemical injury of the thyroid (Capen, 1992) showed that rodents treated with agents that directly interfere with thyroid hormone production in the thyroid gland depress T3 and T4 levels resulting in a compensatory increase of TSH. This TSH stimulation of the thyroid gland leads to hypertrophy, hyperplasia, and neoplasia in rodents. In addition, this excessive secretion of TSH alone, without any chemical exposure, produces a high incidence of thyroid tumors in rodents. Capen concluded that thresholds for agents which inhibit iodide uptake by the thyroid can be established by determining the dose that fails to elicit an elevation in the circulating level of TSH. Hence, the threshold concentration of perchlorate, i.e., the perchlorate concentration below which there is no depression of T3 and T4 accompanied by TSH elevation, is completely protective against carcinogenesis.

Human Data.

Brabant et al conducted a study in which 5 healthy males were exposed to an oral treatment of 300 mg of perchlorate 3 times daily over a 4-week period. Mean serum TSH levels decreased slightly and the thyroid volumes were unaltered. The body weights of the volunteers were not provided. However, using the standard 70 kg default body weight used for risk assessment results in a dose of 12.86 mg/kg/day. This would suggest that the threshold dose for thyroid hormone effects in healthy humans is higher than 12.86 mg/kg/day.

Burgi et al (1974) administered 200 mg of perchlorate 3 times daily to three healthy females and two healthy males for 8 days. The average dose for the females was 11.04 mg/kg/day and the average for the males was 8.42 mg/kg/day. These doses were sufficient to completely block iodide uptake by the thyroid as measured in the urine. However, thyroid hormone levels were not measured in order to determine if this dose produced a decrease in T3 and T4 or an increase in TSH levels.

A study that used potassium perchlorate (KP) to displace iodide from the thyroid gland (Stanbury and Wyngaarden, 1952) was used as the basis for deriving the EPA's provisional RfD. Standbury and Wyngaarden found that 0.14 mg/kg/day (assuming a body weight of 70 kg) was not sufficient to completely block iodide uptake. However, 1.4 mg/kg/day was sufficient to block 90% of the measured iodide. The study did not evaluate the effects of KP on thyroid hormone levels and only three doses were given. These three studies are summarized in Table 1.

Study	Exposure Conditions	Conclusions
Brabant et al	-12.86 mg/kg/day	-TSH levels decreased slightly
Burgi et al	-11.04 mg/kg/day for four weeks (males) -8.22 mg/kg/day for four weeks (females)	-Sufficient to completely block iodide uptake by the thyroid
Stanbury and Wyngaarden	-0.14 mg/kg (once) -1.4 mg/kg (once)	-55% of initially accumulated radioactive iodide was present in the neck -15% of initially accumulated radioactive iodide was present in the neck

Table 1. Summary of human studies using perchlorates.

Animal Data.

With regard to animal data, Shigan conducted a study in which 'white rats' were given AP under various conditions (1963, translated from Russian, 1994). The rats were treated with doses ranging from 2500 to 6500 mg/kg and observed for 15 days. Even though most of the animals died during the first 3 days, Shigan was able to calculate the dose of AP which killed fifty percent of the total experimental population (i.e., the LD_{50}) (see Table 2). 'White rats' were also exposed to AP under two other conditions described in Table 2. However, these results do not provide any insight in determining a threshold dose because there were no doses given at low concentrations.

Exposure Conditions	Conclusions
4200 mg/kg (once)	-LD_{50} value
650 mg/kg/day for one month	-No noticeable cumulative properties
190 mg/kg/day for three months	-Affects the regulation of the involuntary nervous system -Causes a statistically reliable change in the protein fractions of the blood serum -Disrupts the liver's ability to produce glycogen for carbohydrate storage

Table 2. Results of Shigan's Experiments on 'white rats'

Mannisto et al (1979) studied the effects of Potassium Perchlorate (KP) on the thyroid of the Sprague-Dawley rat. He found that doses of KP from 7.8 to 15.3 mg/kg/day administered over a 4 day period reduced serum T3 and T4 levels and increased TSH levels.

These animal experiments do not provide enough information on which to base an accurate RfD since dose-response data on thyroid hormone levels are lacking (e.g., Shigan) or the period of

administration was too short (e.g., Mannisto). Since the EPA based their provisional perchlorate RfD on the Stanbury and Wyngaarden study and predicted that chronic administration of perchlorate at the dose used in that study would likely have resulted in lowering of the patients T3 and T4 levels, with subsequent increases in the levels of TSH, this hypothesis was tested by designing a study in which changes in thyroid hormone levels in the Sprague-Dawley rat would be measured in response to increasing doses of AP (Caldwell and Mattie, 1995). A 14-day pilot study provided specific dose-response data over a wide range of doses, from which a threshold level for thyroid hormone effects of AP could be estimated (Caldwell et al, 1995). Since increased levels of TSH are a sign that the thyroid has been disturbed, if a dose of perchlorate can be found from which there is no observed statistically significant increase in the amount of TSH in the blood, this dose can be considered at or below the threshold dose (Capen, 1992). Subsequently, this dose can be used in deriving a RfD.

PILOT STUDY

Groups of six male and six female Sprague-Dawley rats were dosed with AP in drinking water at concentrations of 0 (control), 1.25, 5.0, 12.5, 25, 50, 125, or 250 mg/L. Animals were sacrificed after fourteen days and thyroid hormone levels were measured using a radioimmune assay technique. The actual dose of AP administered to each animal was calculated by multiplying the concentration of AP administered in the drinking water by each animal's average water consumption over the 14-day period and dividing this number by each animal's average body weight over the 14-day period. Selected thyroid hormone data are presented in Table 3.

RESULTS

General

AP did not have a statistically significant effect on the average water consumption of either sex at the concentrations administered. Nor did AP have a statistically significant effect on the body weight gain of either sex; both sexes gained weight in the same manner over the two-week period.

Thyroid hormone levels

AP had a statistically significant affect on the thyroid hormone levels in both sexes. The T3 and T4 levels decreased while TSH, rT3, and Tg increased with increasing doses of AP in both males and females; however, the sexes were not affected to the same extent.

Dose (mg/kg-d) (male,female)	T3-Male	T3-Female	T4-Male	T4-Female	TSH-Male	TSH-Female
0 (control)	133	129	5.1	5.0	14.5	11.3
0.11, 0.12	124	85	4.8	4.4	15.0	13.1
0.44, 0.47	106	84	4.7	4.1	16.9	14.6
1.11, 1.23	90	81	4.3	4.0	20.2	15.4
2.26, 3.06	76	79	4.2	3.9	30.2	17.4
4.32, 4.91	71	72	4.1	3.7	31.2	19.2
11.44, 11.47	66	69	3.4	3.3	34.0	22.7
22.16, 24.86	66	66	3.0	2.9	37.4	29.9

Table 3. Effects on Thyroid Hormone Levels.

CONCLUSIONS

The data derived from the two-week study show a decrease of T3 and T4 with a concomitant rise in TSH with increasing doses of AP. The NOAEL was 0.44 mg/kg/d and LOAEL was 1.11 mg/kg/d for the male rats and 0.12 and 0.47 mg/kg/d, respectively, for female rats. This is consistent with the literature which shows a higher level of circulating TSH in male rats compared to females (Jubb et al, 1993).

Long-term perturbations of the pituitary-thyroid axis are more likely to predispose laboratory animals to a higher incidence of proliferative lesions than is the case in the human thyroid. This appears to be particularly true in the male rat in which there usually are higher circulating levels of TSH than in females. The greater sensitivity of the animal thyroid to derangement by chemicals and physiologic perturbations also is related to the shorter plasma half-life of T4 (12-24 hours) than in humans (5-9 days), due, in part, to the considerable differences between species in the transport proteins for T4. In humans, circulating T4 is bound primarily to thyroxine-binding globulin (TBG), but this high-affinity binding protein is not present in rodents. T3 is transported bound to TBG and albumin in humans, but only to albumin in rats. In general T3 is bound less avidly to transport proteins than is T4, resulting in a faster turnover and shorter plasma half-life in most species (Jubb et al, 1993).

Although the data established the NOAEL for thyroid hormone effects, more research is needed in order to determine a precisely defined threshold dose for such effects, and to evaluate the dose-response relationship for other potential toxic effects of AP, such as changes in the production of red blood cells (i.e., hematopoiesis). A planned 90-day study will use doses selected to better estimate the threshold dose and to refine the NOAEL for thyroid hormone effects and other toxicity endpoints. The additional data obtained from this subchronic study will support the use of a less conservative uncertainty factor since data gaps on reproductive and developmental toxicity will be filled, the threshold for effects on thyroid hormone homeostatis will be determined, and investigation of hematopoietic effects will be performed. Therefore, a RfD between 1E-3 mg/kg/day and 1E-2 mg/kg/day appears to be reasonably obtainable.

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EFFECTS OF AMMONIUM PERCHLORATE ON THE THYROID HORMONE LEVELS OF THE SPRAGUE-DAWLEY RAT

THESIS

James H. King, Jr.

Presented to the Faculty of the School of Engineering

of the Air Force Institute of Technology

In Partial Fulfillment of the

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Masters of Science in Engineering and Environmental Management

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III. Methodology

Introduction

This section describes the process by which this research was conducted. The design will be explained, followed by a description of the execution and the analysis techniques. It concludes with a summary of the chapter.

Design

The 14-day pilot study included 96 rats, 48 male and 48 female. They were divided into eight dose groups, including a control (Table 3-1). The male rats were estimated to consume water at the rate of 45 ml/day/rat and have an estimated body weight of 450 grams. The female rats were estimated to consume water at the rate of 27 ml/day/rat and have an estimated body weight of 270 grams. The target doses are specified in Table 2. These target doses were designed around an estimated threshold dose of 10 mg/kg/day based on the available literature (Caldwell, 95).

Group	No. of Animals		AP Conc. (mg/L)	AP Target Dose (mg/kg/day)
	Males	Females		
Control	6	6	0.00	0.0
Very Low	6	6	1.25	0.125
Low	6	6	5.00	0.5
Med. Low	6	6	12.50	1.25
Medium	6	6	25.00	2.5
Med. High	6	6	50.00	5.0
High	6	6	125.00	12.5
Very High	6	6	250.00	25.0

Table 3-1. Pilot Study Dose Groups, Concentrations and Target Doses
Source: Caldwell (1995).

Execution

The study began with 100 rats, 50 male and 50 female. These rats were single housed throughout the study. The quarantine period lasted for 14 days. During the last 7 days of quarantine, the water consumption rates and body weights were measured as baseline data. On the last day of the quarantine the rats were randomly assigned, using the PATH/TOX (XMSC, 1993) randomization algorithm, to 8 groups, each containing 6 males and 6 females.

The dosing solutions were prepared in eight 20 liter containers on day 14 of the quarantine and samples were taken to verify accurate concentrations and stability. The dosing period began the day following the quarantine period. The animals were dosed for 14 days according to table 3-1. The animals were weighed on days 8 and 14 of the quarantine and days 7 and 15 or 16 of the study. Water consumption was measured on day 12 of the quarantine and days 1, 4, 7, 10 and 14 of the study and averaged over the respective periods. This data was used to determine if the dosing solutions affected water consumption or body weight, and calculate the actual dose consumed by the animals.

Following the dosing period, the rats were euthanised via CO_2 inhalation on day 15 or 16. Blood was immediately drawn from the vena cava at necropsy and centrifuged. The serum was stored at -20° C until analyzed for the hormone levels listed above using commercially available radioimmunoassay kits.

Statistical Analysis

The results were analyzed using two-factor Analysis of Variance (ANOVA) (Statistix 4.1), Multivariate Analysis of Variance (MANOVA) (SAS), Tukey's method for multiple comparisons (Statistix 4.1), and Maximum Likelihood Estimation (MLE) (SAS). Two-factor ANOVA was used to determine if there were statistically significant differences between dose groups and the male and female rats. Two-factor MANOVA was used to determine if there were statistically significant differences between dose groups and the male and female rats. If the males and females responded similarly to the dosing, Tukey's method for multiple comparisons was used to determine which dose groups differed.

Capen (1992) concluded that if a dose can be found for which there is no decrease in T3 or T4 accompanied by an increase in TSH, that dose can be considered the threshold. Therefore, the sigmoid function was used to fit the data points for T3 and TSH. The sigmoid function was not used to fit the T4 data because there was no dose response relationship. Maximum Likelihood Estimation (MLE) was then used to determine the parameter point estimates for the sigmoid function. Once the parameter estimates were determined, the function was evaluated using the F-test for lack of fit (LOF). If the functions passed the LOF test, this would mean that they accurately characterized the relationship between dose and hormone levels. These functions, which serve as conservative estimations of the dose-response relationship, could subsequently be used to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group.

Two-Factor ANOVA (Devore, 1991):

The two-factor ANOVA was used to determine whether there were statistically significant differences between dose/sex group means. This is done by first determining the grand mean (the mean of all dose/sex groups) and then comparing each data value to the grand mean. The differences between each data value and the grand mean are then squared and summed to determine the sum of squares for the total samples (SST). Next, the mean for each dose group is determined and compared against the grand mean. The differences are squared and summed to determine the sums of squares for dose group (factor A, SSA). SSB (factor B), or the sums of squares for the sex factor, is determined by holding the dose factor constant and determining the mean across sex for each sex and comparing them to the grand mean. SSAB (interaction sum of squares) is determined by computing the mean for each dose/sex group and subtracting the means of the dose groups and sexes and adding the grand mean. SSE (error sum of squares) is determined by each data value to the dose/sex mean. Each sums of squares is then divided by its degrees of freedom to obtain the mean square for each factor (e.g. MSA, MSB, MSAB). These mean square values are divided by the mean square error (MSE) to determine the f-ratio (f has a certain distribution when the null hypothesis is true). If the computed f-value is greater than the value chosen for alpha (the level at which any percentage above is grounds to reject the null hypothesis and subject to type-I error or the error of rejecting the null when it is true), then the data cannot be considered to be from the distribution and the null hypothesis is rejected. The statistical analysis package Statistix 4.1 was used to perform the two-factor ANOVA. The research problems were as follows:

Problem 1 (Average Water Consumption)

(1) Does the dose of AP affect average water consumption?

(2) Does the sex of the rat affect average water consumption?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Problem 2 (Body Weight Gain)

(1) Does the dose of AP affect average body weight gain?

(2) Does the sex of the rat affect average body weight gain?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Problem 3 (Thyroid/Body Weight Ratio)

(1) Does the dose of AP affect thyroid/body weight ratio?

(2) Does the sex of the rat affect thyroid/body weight ratio?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Research Null Hypotheses:

Hypothesis 1 (Average Water Consumption)

(1) The dose of AP does not affect average water consumption.

(2) The sex of the rat does not affect average water consumption.

(3) There is no interaction between the dose of AP and the sex of the rat.

Hypothesis 2 (Body Weight Gain)

(1) The dose of AP does not affect body weight gain.

(2) The sex of the rat does not affect body weight gain.

(3) There is no interaction between the dose of AP and the sex of the rat.

Hypothesis 3 (Thyroid/Body Weight Ratio)

(1) The dose of AP does not affect thyroid/body weight ratio.

(2) The sex of the rat does not affect thyroid/body weight ratio.

(3) There is no interaction between the dose of AP and the sex of the rat.

An answer which contradicted any one of the previous hypotheses resulted in rejecting the respective null hypothesis in favor of the alternate hypothesis which states that there is an affect.

Two-Factor MANOVA (Barcikowski, 1983; Hair et al., 1979):

Two-factor MANOVA is similar to ANOVA, except there are several dependent variables (e.g. T3, T4, TSH, rT3 and Tg) instead of one dependent variable (e.g. average water consumption or body weight gain). While ANOVA and MANOVA both test for differences among groups using sums-of-squares, MANOVA finds them using the covariance structure of the dependent variables. The F-ratio tests for equality among dose groups based on their vector means. The statistical analysis package SAS was used to perform the MANOVA. The research problem was as follows:

Problem

(1) Does the dose of AP affect Tg, T3, rT3, T4, and TSH thyroid hormone levels?

(2) Does the sex of the rat affect Tg, T3, rT3, T4, and TSH thyroid hormone levels?

(3) Is there any interaction between the dose of AP and the sex of the rat?

Research Null Hypotheses

(1) The dose of AP has no affect on Tg, T3, rT3, T4, and TSH thyroid hormone levels.

(2) The sex of the rat has no affect Tg, T3, rT3, T4, and TSH thyroid hormone levels.

(3) There is no interaction between the dose of AP and the sex of the rat.

An answer which contradicted any of the three previous null hypotheses resulted in rejecting the respective null hypothesis.

Maximum Likelihood Estimation

Maximum likelihood estimation (MLE) was used to obtain point estimates of the parameters of the sigmoid function. The sigmoid function was used because it approaches a threshold affect at low doses (Fig. 3-1). The sigmoid function is as follows:

$$\text{HormLevel}(\text{Dose}, B0, B1) = \frac{B0 \cdot \text{Dose}^{B1}}{\left[\text{Dose}^{B1} + \left(\frac{B0}{2}\right)^{B1}\right]}$$

Where B0 is the highest observed response value (highest observed hormone level) and B1 is an exponent parameter which controls the function shape.

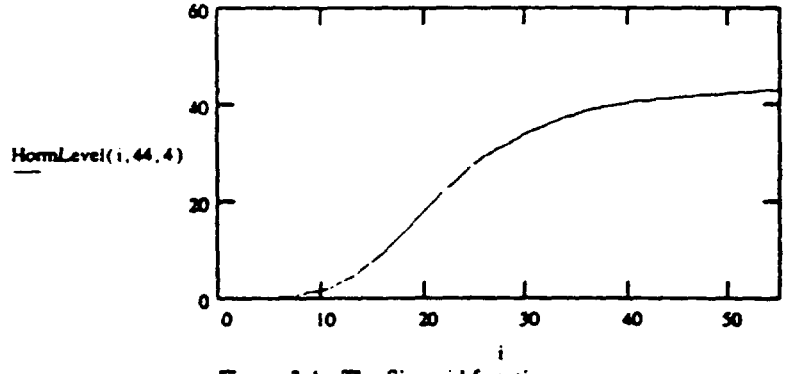

Figure 3-1. The Sigmoid function.

The MLE method assumes that all the hormone levels are normally distributed, have equal variances and are independent. The likelihood function:

$$L(\mu_i,\sigma) = \prod_{i=1}^{n} \frac{1}{(2\cdot\pi\cdot\sigma^2)^{\frac{1}{2}}} e^{\left[-\frac{1}{2\cdot\sigma^2}\cdot(Y_i-\mu_i)^2\right]}$$

where the expected value of the sigmoid function, $E(f(x_i,B0,B1)) = \mu_i$ (the population mean) and the expected value of the difference between each observed value of hormone level (y_i) and the μ_i squared, $E(y_i - \mu_i)^2 = \sigma^2$. The expected value of the sigmoid function is substituted in the likelihood function for μ_i and $E(y_i - \mu_i)^2$ for σ^2. Subsequently, the values of B0 and B1 that maximize the likelihood function are the maximum likelihood estimators.

Once the maximum likelihood estimators are determined, the sigmoid function can be tested to see how well it characterizes the data with an F-test for lack of fit (LOF). Once the function passes the LOF test, it can be used to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group.

F Test for Lack of Fit

The F test for lack of fit assumes that the observations Y (e.g. hormone levels) for given X (e.g. dose) are independent and normally distributed, and the distributions of Y have the same variance. The test is accomplished by first determining the pure error sum of squares (SSPE) and the residual sum of squares (SSRes) which are computed in the ANOVA table. The difference between these two error sums of squares is called the lack of fit sum of squares (SSLF). After dividing the SSPE and SSLF by their appropriate degrees of freedom, the F statistic can then be expressed as follows:

$$F = \frac{MSLF}{MSPE}$$

The research problem for each data set was as follows:

Problem

Does the function accurately characterize the data?

Research Null Hypothesis

The function accurately characterizes the data.

If the value for the computed F is less than or equal to the critical value for the level of significance (α=.05), then the null hypothesis holds.

Tukey's Method for Multiple Comparisons

When the no-interaction hypothesis was not rejected and at least one of the two main effect null hypotheses was rejected, Tukey's method was used to identify significant differences between dose groups. For identifying differences among the means when the null hypothesis was rejected,

1. Obtain the value of the upper-tail α from the studentized t-distribution, above which the null is rejected (Q).

2. Compute $w = Q * (MSE/(JK))^{1/2}$, where MSE is the mean squared error obtained from the ANOVA table and JK is the number of observations averaged to obtain each of the sample means compared in step 3.

3. Order the sample means from smallest to largest and underscore all pairs that differ by less than w. Pairs not underscored correspond to significantly different levels for the factor under consideration.

Summary

To determine whether there was a dose-response relationship for body weight gain, average water consumption and thyroid/body weight ratio, two-factor ANOVA was used. If no interaction was observed, Tukey's method for multiple comparisons was used to determine which groups differed by dose. Two-factor MANOVA was used to determine statistically significant differences between dose groups with five dependent variables. MLE was then used to find the point estimates for the parameters of the sigmoid curve which was used to fit the data. Once the parameters which maximized the likelihood function were determined, the F-test for lack of fit was used to determine if the functions accurately characterized the data sets. The results are presented in Chapter 4.

IV. Data Description and Analysis

Introduction

This chapter presents and analyzes the raw data obtained from this study. Two-factor ANOVA was used to determine statistically significant differences between dose groups based on weight gain, water consumption, thyroid/body weight ratio. Two-factor MANOVA was used to determine significant differences between dose groups and sex based on thyroid hormone levels. When a null hypothesis was not rejected the analysis was terminated. However, when the no-interaction hypothesis was not rejected and at least one of the two main effect null hypotheses was rejected, Tukey's method was used to identify which levels differed from the control. Maximum Likelihood Estimation (MLE) was then used to determine the point estimates for the parameters of the sigmoid function used to fit the data. The F-test for lack-of-fit was then used to determine if the function accurately characterized the data.

The data was analyzed in this order. First, the water consumption data was analyzed to determine whether there was any statistically significant difference between dose groups and what the actual doses were. Next, body weight gain and thyroid/body weight ratio was analyzed to determine whether there was a difference between dose groups. The effect of dose, if any, on thyroid hormone levels was then evaluated. If an affect was noted, maximum likelihood estimation was used to maximize the parameters for the sigmoid function. The function was then used to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group.

Water Consumption and Dose

The average water consumed by dose group is shown in figures 4-1 and 4-2. Two-factor ANOVA revealed that there were no statistically significant differences

ANALYSIS OF VARIANCE TABLE FOR AVCONSMP

SOURCE	DF	SS	MS	F	P
GROUP (A)	7	280.870	40.1243	1.07	0.3900
SEX (B)	1	4423.51	4423.51	118.07	0.0000
A*B	7	195.063	27.8662	0.74	0.6372
RESIDUAL	80	2997.26	37.4658		
TOTAL	95	7896.71			

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF AVCONSMP BY GROUP

DOSE GROUP	MEAN	HOMOGENEOUS GROUPS
5	37.612	I
4	33.929	I
8	33.883	I
2	33.622	I
7	33.522	I
6	33.337	I
3	32.667	I
1	31.097	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL Q VALUE	4.401	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	7.7762		
STANDARD ERROR FOR COMPARISON	2.4989		
ERROR TERM USED: RESIDUAL, 80 DF			

Table 4-1. Results of Two-factor ANOVA for average water consumption.

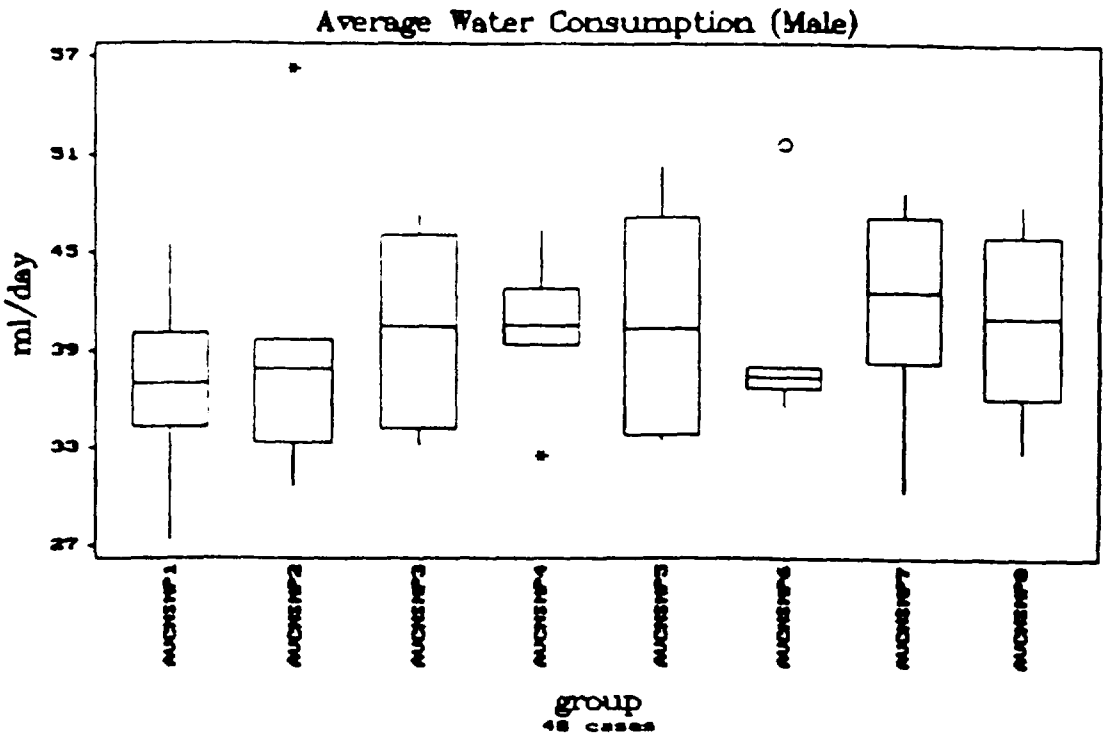

Figure 4-1. Boxplots of male rat average water consumption by dose group.

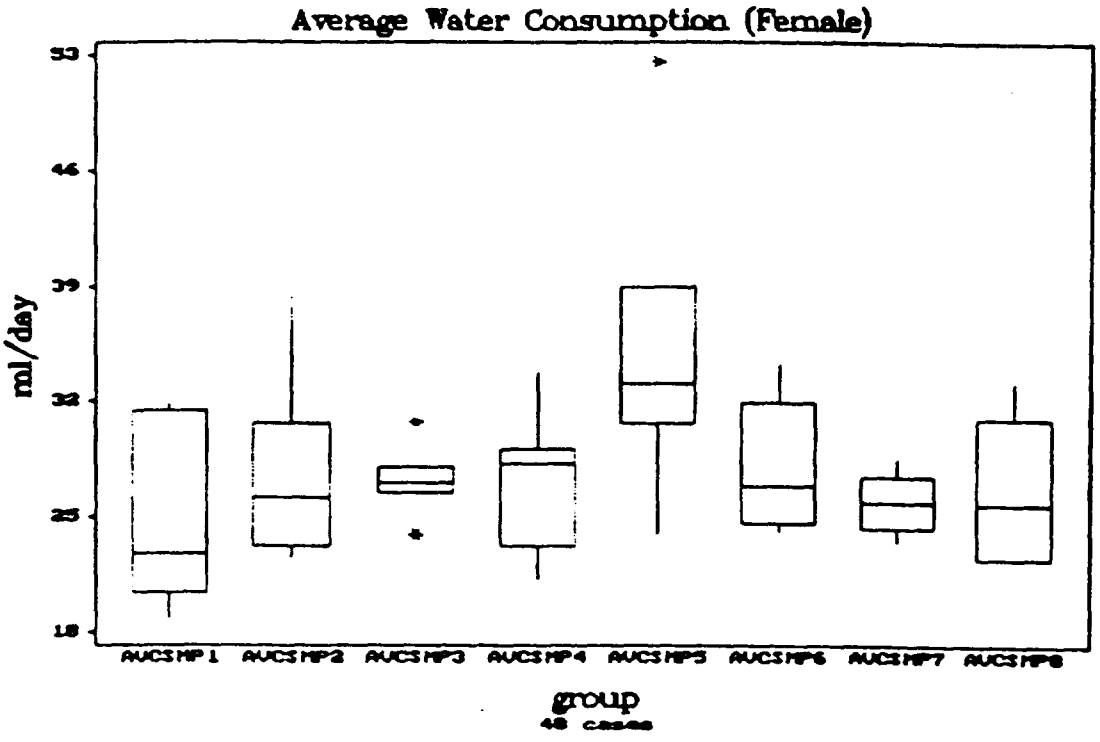

Figure 4-2. Boxplots of female rat average water consumption by dose group.

between the groups with regard to dose, indicating that the concentration of AP in the water was not sufficient to decrease the consumption rate. However there were differences with regard to sex (Table 4-1). The P value for sex was much less than .05 indicating that the male consumption rate is different from that of the female. The average water consumption was used to determine the actual dose administered (Table 4-2).

Group	No. of Animals Males	Females	AP Conc. (mg/L)	AP Target Dose (mg/kg/day)	AP Dose (mg/kg/day) Males	Females
Control	6	6	0.00	0.0	0.0	0.0
Very Low	6	6	1.25	0.125	0.110	0.124
Low	6	6	5.00	0.5	0.443	0.466
Med. Low	6	6	12.50	1.25	1.112	1.232
Medium	6	6	25.00	2.5	2.263	3.063
Med. High	6	6	50.00	5.0	4.321	4.912
High	6	6	125.00	12.5	11.443	11.469
Very High	6	6	250.00	25.0	22.157	24.863

TABLE 4-2. Ammonium perchlorate dose by group.

Body Weight Gain

The body weight gained per dose group is shown in figures 4-3 and 4-4. The two-factor ANOVA indicated that there were no statistically significant differences between dose groups, indicating that the concentrations of AP did not affect appetite. However there were differences with regard to sex. The P value of .0001 for sex was much less than α=.05. The male rats gained more weight than the female rats. No interaction between dose and sex was observed indicating that both sexes responded in the same way to the dosing.

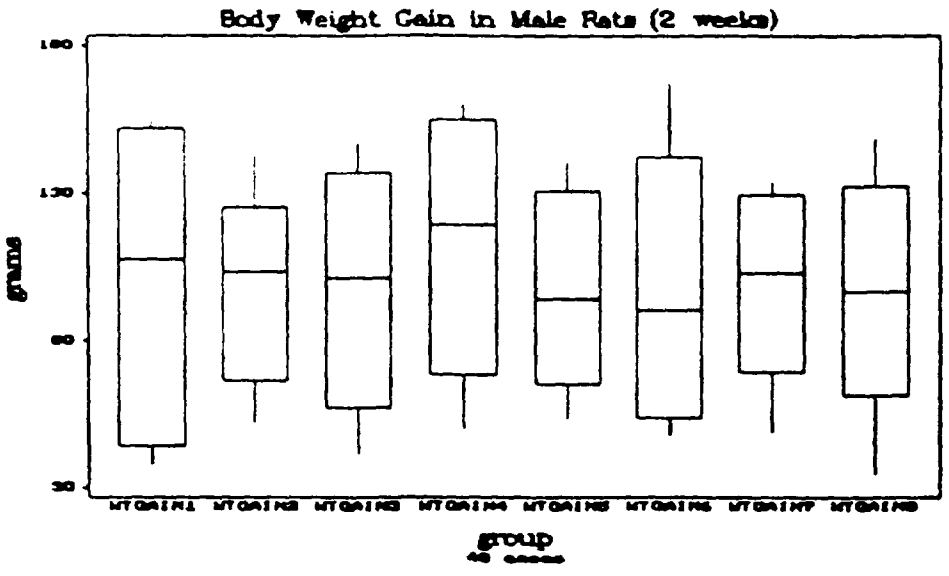

Figure 4-3. Boxplots of male rat body weight gained by dose group.

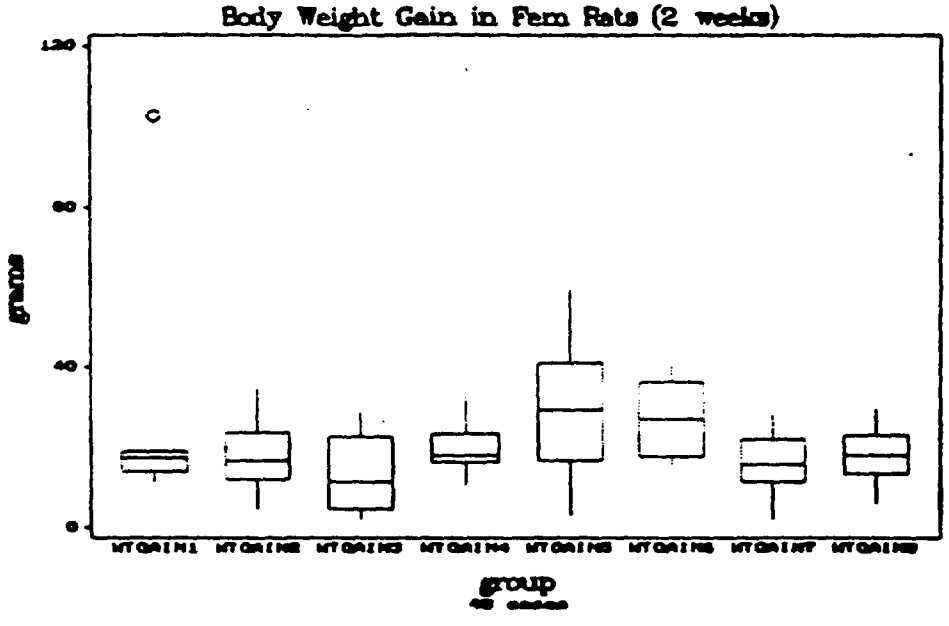

Figure 4-4. Boxplots of female rat body weight gained by dose group.

ANALYSIS OF VARIANCE TABLE FOR WTGAIN

SOURCE	DF	SS	MS	F	P
SEX (A)	1	1.463E+05	1.463E+05	129.86	0.0000
GROUP (B)	7	1515.72	216.532	0.19	0.9851
A*B	7	1476.96	210.995	0.19	0.9861
RESIDUAL	80	90147.9	1126.85		
TOTAL	95	2.395E+05			

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF WTGAIN BY GROUP

DOSE GROUP	MEAN	HOMOGENEOUS GROUPS
4	66.033	I
1	65.608	I
5	63.192	I
6	63.000	I
2	58.575	I
7	56.883	I
8	56.575	I
3	55.583	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL Q VALUE	4.401	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	42.646		
STANDARD ERROR FOR COMPARISON	13.704		

ERROR TERM USED: RESIDUAL, 80 DF

Table 4-3. Results of Two-factor ANOVA for body weight gain.

Thyroid/Body Weight Ratio

Thyroid/body weight ratio data are shown in figures 4-5 and 4-6. Two-factor ANOVA indicated that there were differences among the means with respect to dose and sex. No interaction was observed indicating that males and females reacted similarly to the dosing (Table 4-4). Tukey's test for multiple comparisons revealed that the ratio for dose groups 7 and 8 increased and were statistically significantly different from the control group (Table 4-4).

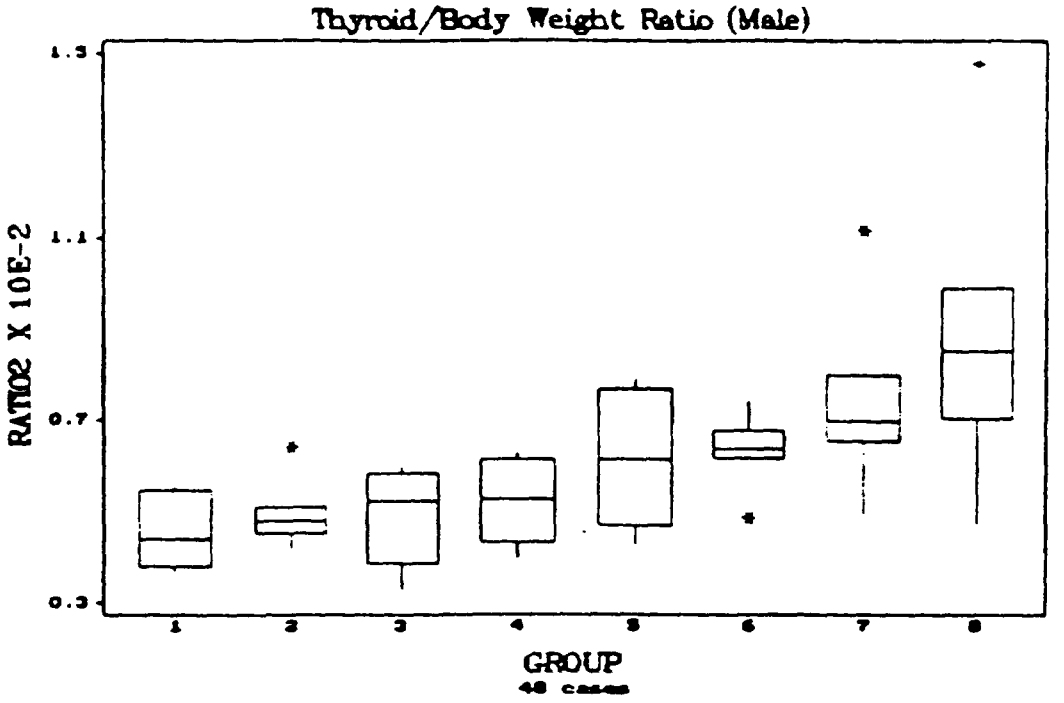

Figure 4-5. Boxplots of male thyroid/body weight ratios by dose group.

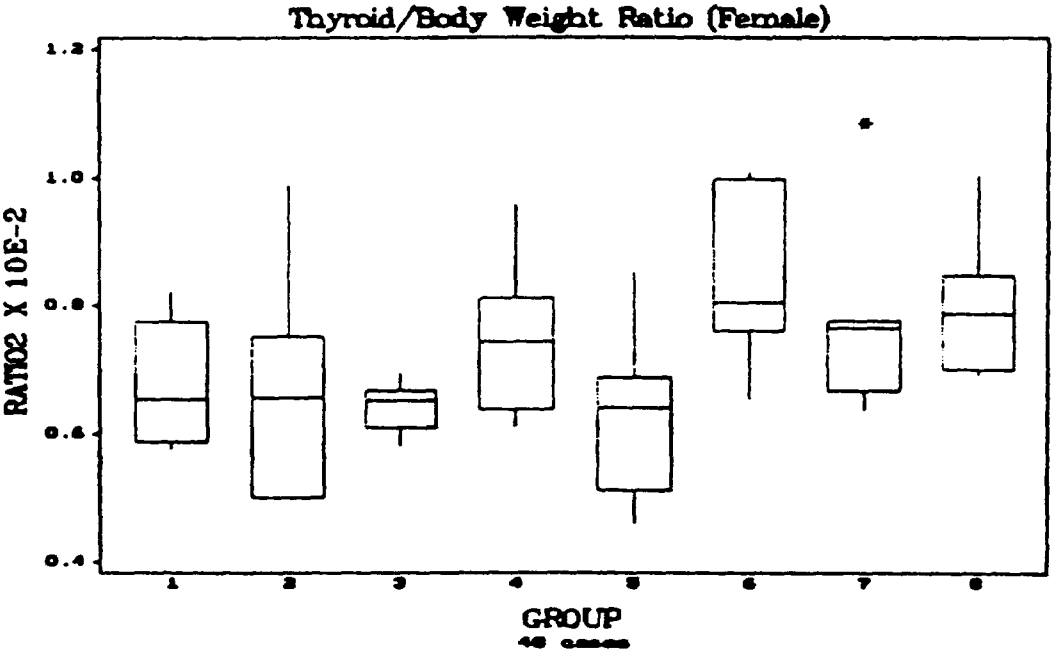

Figure 4-6. Boxplots of female thyroid/body weight ratios by dose group.

ANALYSIS OF VARIANCE TABLE FOR RATIO

SOURCE	DF	SS	MS	F	P
GROUP (A)	7	9.128E-07	1.304E-07	5.76	0.0000
SEX (B)	1	3.425E-07	3.425E-07	15.14	0.0002
A*B	7	2.841E-07	4.059E-08	1.79	0.0992
RESIDUAL	80	1.810E-06	2.262E-08		
TOTAL	95	3.349E-06			

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF RATIO BY GROUP

DOSE GROUP	MEAN	HOMOGENEOUS GROUPS
8	8.47E-04	I
7	7.64E-04	II
6	7.36E-04	III
4	6.36E-04	.II
5	6.24E-04	.II
2	5.87E-04	.II
3	5.67E-04	...I
1	5.66E-04	...I

THERE ARE 3 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.
CRITICAL Q VALUE 4.401 REJECTION LEVEL 0.050
CRITICAL VALUE FOR COMPARISON 1.911E-04
STANDARD ERROR FOR COMPARISON 6.140E-05
ERROR TERM USED: RESIDUAL, 80 DF

Table 4-4. Results of two-factor ANOVA for thyroid/body weight ratio.

Thyroid Hormone Levels

Two-factor MANOVA was used to determine relationships between thyroid hormone levels. The results of the MANOVA are condensed in table 4-5.

Thyroid Hormone	P-Value (Dose)	P-Value (Sex)	P-Value (Dose*Sex)
Tg	.0001	.2321	.0001
rT3	.0001	.6923	.4104
T3	.0001	.0001	.0001
TSH	.0001	.0001	.0001
T4	.0006	.0001	.2909

Table 4-5. Results of two-factor MANOVA.

The results show that the null hypothesis for dose is rejected for every hormone indicating that dose does have a statistically significant impact on their levels. The sexes within dose groups were statistically significantly different in T3, TSH, and T4. In addition, the null hypotheses for interaction for Tg, T3 and TSH were rejected, indicating that the sexes were not similarly affected by the dosing. Therefore, MANOVA was used to evaluate the sexes separately.

Males

The correlation matrix for males was as follows (Fig. 4-7) (SAS output):

Correlation Analysis/Pearson Correlation Coefficients

	HTG	RT3	T3	TSH	T4
HTG	1.00000				
	0.0				
RT3	0.82190	1.00000			
	0.0001	0.0			
T3	-0.77049	-0.76134	1.00000		
	0.0001	0.0001	0.0		
TSH	0.83526	0.80132	-0.88439	1.00000	
	0.0001	0.0001	0.0001	0.0	
T4	-0.45956	-0.35538	0.21192	-0.34479	1.00000
	0.0010	0.0132	0.1482	0.0164	0.0

Figure 4-7. Correlation matrix for male rats.

The correlation matrix showed a strong negative relationship between T3 and TSH. This supports the literature findings.

Females

The correlation matrix for females was as follows (SAS output):

Correlation Analysis/Pearson Correlation Coefficients

	HTG	RT3	T3	TSH	T4
HTG	1.00000				
	0.0				
RT3	0.66152	1.00000			
	0.0001	0.0			
T3	-0.74760	-0.62200	1.00000		
	0.0001	0.0001	0.0		
TSH	0.95962	0.65659	-0.67418	1.00000	
	0.0001	0.0001	0.0001	0.0	
T4	-0.00165	0.14767	-0.20017	-0.02562	1.00000
	0.9911	0.3165	0.1725	0.8627	0.0

Figure 4-8. Correlation matrix for female rats.

The correlation matrix for females showed a strong negative relationship between T3 and TSH. This also supports the literature findings.

The MANOVA revealed that there was a statistically significant relationship between dose and hormone levels and that the sexes were affected differently. Based on this information sigmoid functions were used to fit the data as closely as possible. These models were then tested for lack of fit.

All the thyroid hormone levels were affected by dose (Table 4-5). Capen (1992) stated that the lowest dose which lowers T3 and/or T4 and simultaneously increases TSH could be considered the threshold dose. Therefore, although all the hormone levels are discussed in the next few sections, only those functions used to fit the data for T3 and TSH will be discussed. T4 showed a statistically significant affect from dose, but there was no dose-response relationship.

Thyroglobulin (Tg)

Tg increased consistently with dose (Figs. 4-9 and 4-10). Since iodized Tg is needed to make MIT and DIT, which combine to form T3 and T4, the negative feedback mechanism could have triggered a response to produce more Tg based on declining T3 levels.

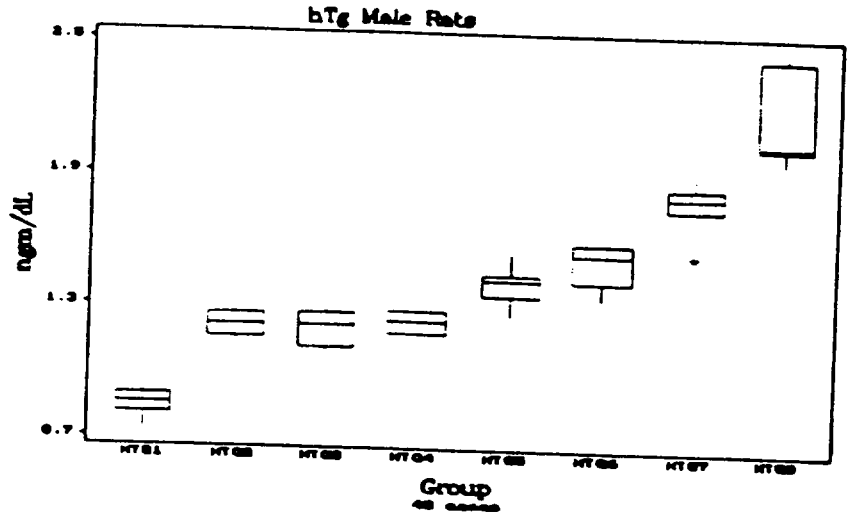

Figure 4-9. Boxplots of male Tg levels by dose group.

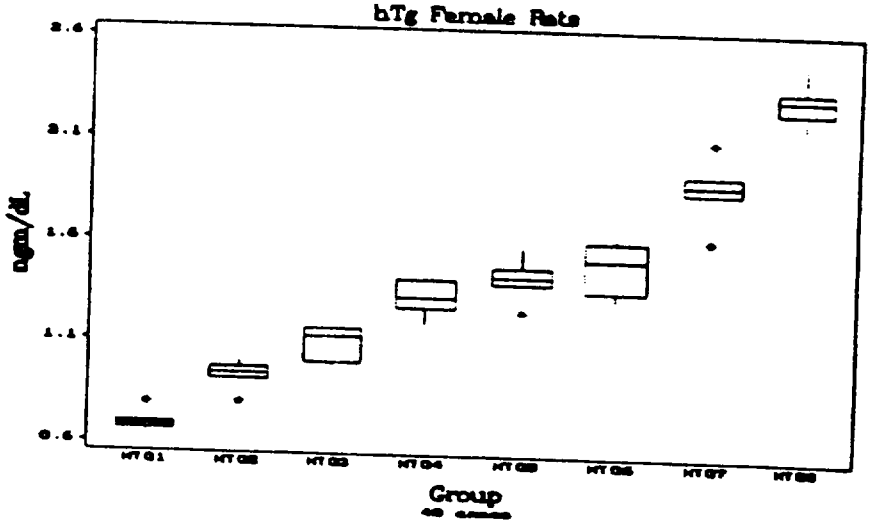

Figure 4-10. Boxplots of female Tg levels by dose group.

Reverse Triiodothyronine (rT3)

rT3 also increased consistently with dose (Figs. 4-11 and 4-12). Since rT3 is formed from T4 in the peripheral tissues and has no known function, except to prevent the formation of T3. An increase in rT3 could have contributed to the depletion of T3.

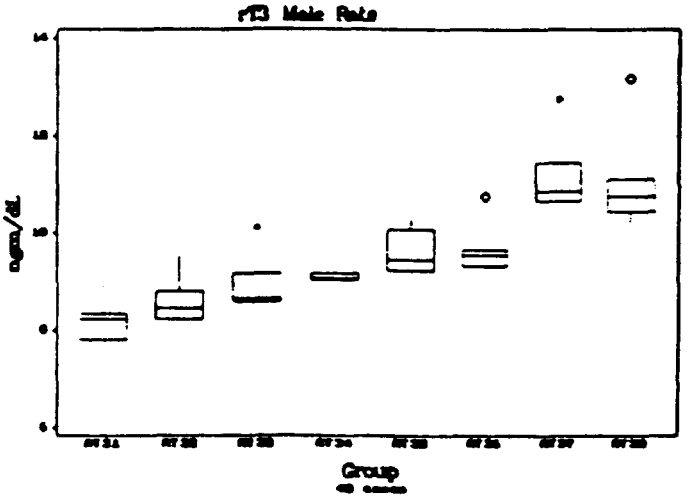

Figure 4-11. Boxplots of male rT3 levels by dose group.

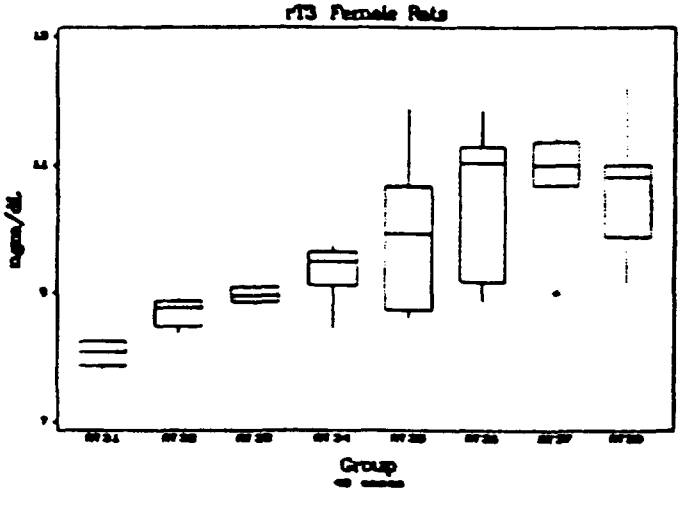

Figure 4-12. Boxplots of female rT3 levels by dose group.

Thyroxine (T4)

Although T4 showed a statistically significant effect, there was no dose-response relationship (Figs. 4-13 and 4-14).

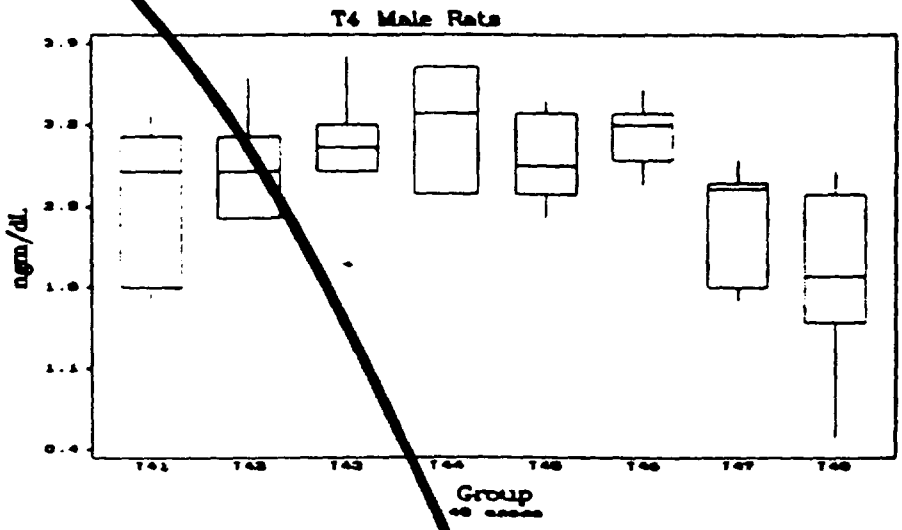

Figure 4-13. Boxplots of male T4 levels by dose group.

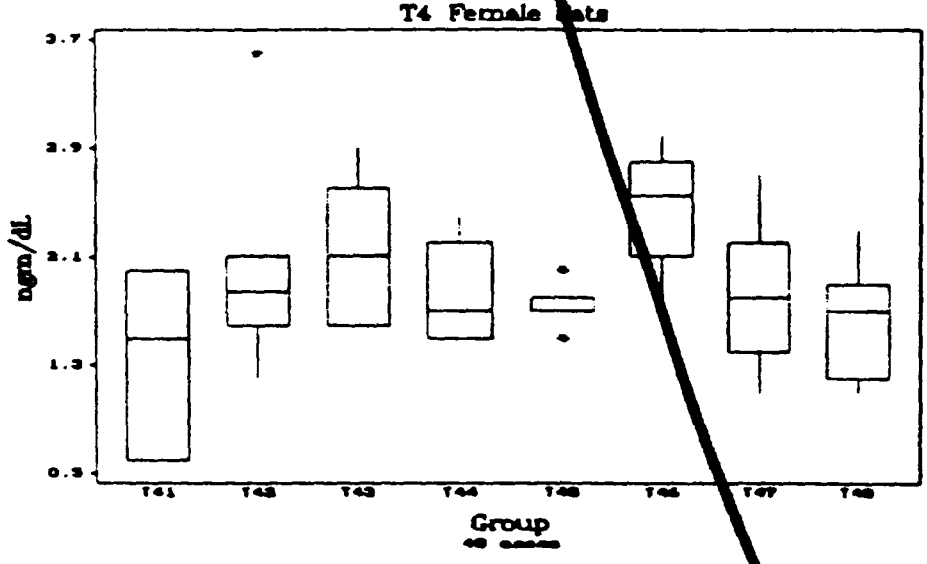

Figure 4-14. Boxplots of female T4 levels by dose group.

Triiodothyronine (T3)

T3 decreased with dose (Figs. 4-15 and 4-16). The mean value for T3 in the control group for females was 128.51 ngm/ml with a standard deviation of 8.99. The mean value for T3 in the control group for males was 132.87 ngm/ml for females with a standard deviation of 11.71.

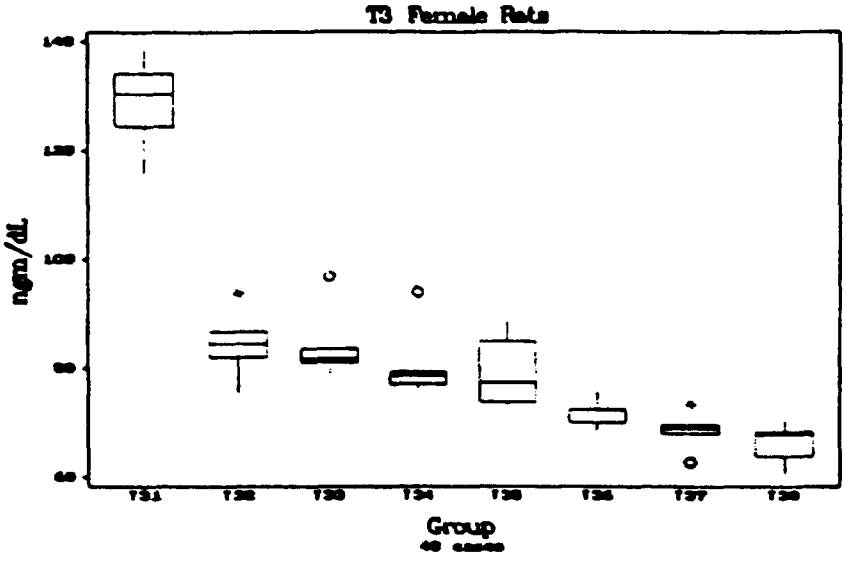

Figure 4-15. Boxplots of female T3 levels by dose group.

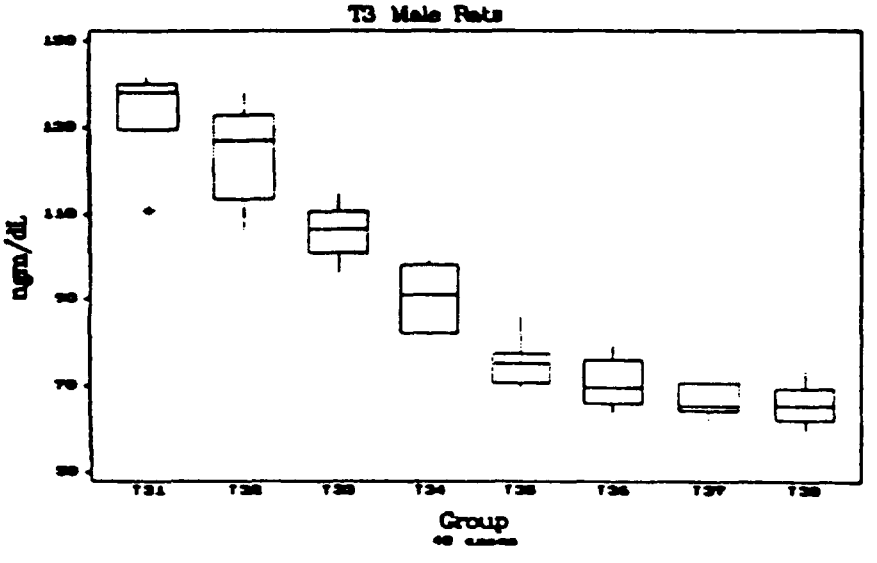

Figure 4-16. Boxplots of male T3 levels by dose group.

Females

The MLE sigmoid function for female T3 levels was as follows:

$$T3Female(Dose) = 138.364 - \frac{132.05\,Dose^{119}}{\left[Dose^{119} + \left(\frac{132.05}{2}\right)^{119}\right]}$$

The relationship of the function to the data is displayed in figure 4-17.

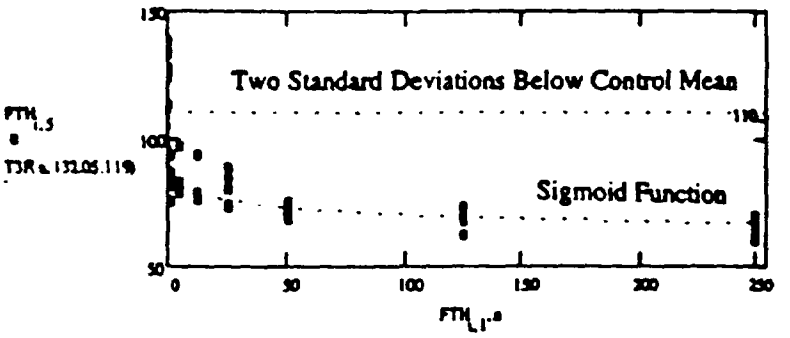

Figure 4-17. T3 dose-response data for females with fitted sigmoid function.

The lack of fit test for the female T3 sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha,v1,v2}$
SS Pure Error	40	1369.845284	34.2461321	3.709	2.34
SS Lack of Fit	6	762.156736	127.026		
SS Error	46	2132.00202			

Table 4-6. Table for T3 sigmoid function lack of fit test (females)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 3.709 > 2.34, the null hypothesis was rejected in favor of the alternate. Therefore, the function did not accurately characterize the data and could not be used to obtain a 95% confidence interval.

Males

The MLE sigmoid function for male T3 levels was as follows:

$$T3Male(Dose) = 141.536 - \frac{136.68\,Dose^{.359}}{\left[Dose^{.359} + \left(\frac{136.68}{2}\right)^{.359}\right]}$$

The relationship of the function to the data is displayed in figures 4-18.

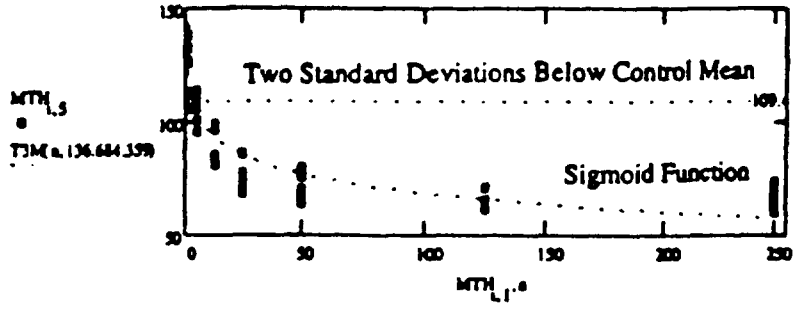

Figure 4-18. T3 dose-response data for males with fitted sigmoid function.

The lack of fit test for the male T3 sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha, v1, v2}$
SS Pure Error	40	2529.145352	63.2286	5.96	2.34
SS Lack of Fit	6	2262.14176	377.0236		
SS Error	46	4791.28711			

Table 4-7. Table for T3 sigmoid function lack of fit test (males)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 5.96 > 2.34, the null hypothesis was rejected in favor of the alternate. As with the females, the function did not accurately characterize the data and could not be used to calculate an upper 95% confidence interval.

Thyroid Stimulating Hormone (TSH)

A very clear relationship between dose and TSH levels was observed (Figs. 4-19 and 4-20). The mean value for TSH in the control group for females was 11.251 ngm/ml with a standard deviation of .4780. The mean value for TSH in the control group for males was 14.472 ngm/ml with a standard deviation of 1.1547.

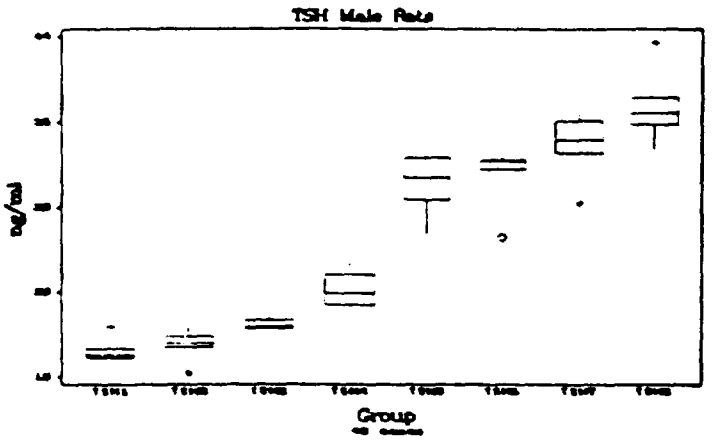

Figure 4-19. Boxplots of male TSH levels by dose group.

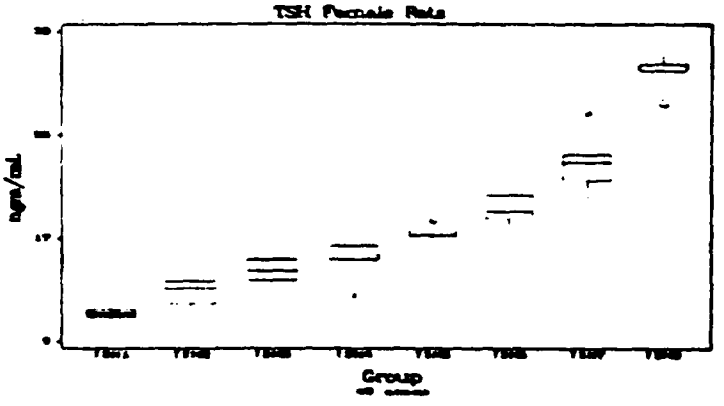

Figure 4-20. Boxplots of female TSH levels by dose group.

Females

The MLE sigmoidal function for female TSH is as follows:

$$TSHFemale(Dose) = 11.248 + \frac{12.885\,Dose^{.7025}}{\left[Dose^{.7025} + \left(\frac{12.885}{2}\right)^{.7025}\right]}$$

The relationship of the function to the data is displayed in figure 4-21.

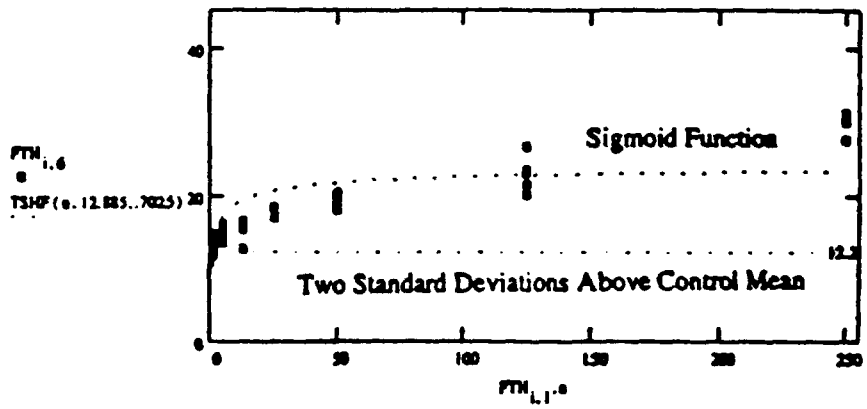

Figure 4-21. TSH dose-response data for females with fitted sigmoid function.

The lack of fit test for the female TSH sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha,v1,v2}$
SS Pure Error	40	60.9892939	1.5247	54.72	2.34
SS Lack of Fit	6	500.556979	83.4261632		
SS Error	46	561.5462732			

Table 4-8. Table for TSH sigmoid function lack of fit test (females)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 54.72 > 2.34, the null hypothesis was rejected in favor of the alternate. Once again, the function did not accurately characterize the data. This time it was soundly rejected.

Males

The sigmoidal function for male TSH is as follows:

$$TSHMale(Dose) = 14.473 + \frac{20.257\,Dose^{1.417}}{\left[Dose^{1.417} + \left(\frac{20.257}{2}\right)^{1.417}\right]}$$

The relationship of the function to the data is displayed in figure 4-22.

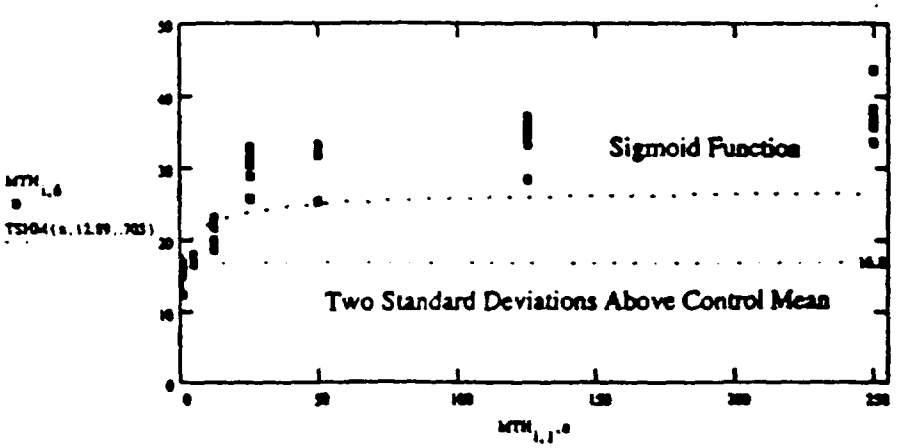

Figure 4-22. TSH dose-response data for males with fitted sigmoid function.

The lack of fit test for the male TSH sigmoid function was as follows:

	DF	SS	MS	$F_{critical}$	$F_{\alpha, v1, v2}$
SS Pure Error	40	218.8194058	11.911	10.0262	2.34
SS Lack of Fit	6	329.090418	54.848403		
SS Error	46	547.9098236			

Table 4-9. Table for TSH sigmoid function lack of fit test (males)

The value for $F_{critical}$ was computed by dividing the Mean Square Lack of Fit by the MS Pure Error. Since 10.02 > 2.34, the null hypothesis was rejected in favor of the alternate. Therefore, the function did not accurately characterize the data.

An upper 95% confidence interval for the sigmoid function could not be determined because the functions were not able to accurately characterize the data.

Therefore, a threshold value could not be determined with the data from this study. However, Tukey's method for multiple comparisons was performed in order to determine the NOAEL values for T3 and TSH. The results are as follows:

T3

Tukey's test for multiple comparisons for female T3 hormone levels revealed that group one was statistically significantly different from the other dose groups (Table 4-10).

STATISTIX 4.1 HORMFEM

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF T3 BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
0	128.51	I
1	84.600	_I
5	84.074	_I
12	80.676	_II
25	79.300	_III
50	71.860	__III
125	68.548	___II
250	66.401	____I

THERE ARE 5 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	10.799		
STANDARD ERROR FOR COMPARISON	3.3787		

Table 4-10. Tukey's test for multiple comparisons for female T3 levels.

Therefore, the NOAEL in this experiment for T3 in female rats is the control.

Tukey's test for multiple comparisons for male T3 levels revealed that groups one and two were statistically the same (Table 4-11).

STATISTIX 4.1 HORMMALE

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF T3 BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
0	132.87	I
1	124.02	I
5	105.67	_I
12	90.459	__I
25	75.417	___I
50	70.690	___I
125	66.465	___I
250	65.936	___I

THERE ARE 4 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	14.674		
STANDARD ERROR FOR COMPARISON	4.5909		

Table 4-11. Tukey's test for multiple comparisons for male T3 levels.

The NOAEL in this experiment for T3 in male rats was .11 mg/kg/day.

TSH

Tukey's test for multiple comparisons for female rats revealed that groups one and two were statistically the same (Table 4-12).

STATISTIX 4.1 HORMFEM

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF TSH BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
250	29.926	I
125	22.905	_I
50	19.254	_I
25	17.385	_II
12	15.358	__II
5	14.584	___II
1	13.051	____II
0	11.251	_____I

THERE ARE 7 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	2.2787		
STANDARD ERROR FOR COMPARISON	0.7129		

Table 4-12. Tukey's test for multiple comparisons for female TSH levels

Therefore, the NOAEL in this experiment for TSH in female rats is .124 mg/kg/day.

Tukey's test for multiple comparisons for male rats revealed that dose groups one, two and three were statistically the same (Table 4-13).

STATISTIX 4.1 HORMMALE

TUKEY (HSD) PAIRWISE COMPARISONS OF MEANS OF TSH BY DOSE

DOSE	MEAN	HOMOGENEOUS GROUPS
250	37.444	I
125	33.960	II
50	31.147	.I
25	30.236	.I
12	20.250	..I
5	16.919	..II
1	15.022	...I
0	14.472	...I

THERE ARE 4 GROUPS IN WHICH THE MEANS ARE
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE	4.520	REJECTION LEVEL	0.050
CRITICAL VALUE FOR COMPARISON	4.3162		
STANDARD ERROR FOR COMPARISON	1.3504		

Table 4-13. Tukey's test for multiple comparisons for male TSH levels.

Therefore, the NOAEL in this experiment for TSH in male rats is .44 mg/kg/day.

A summary of the NOAELs is presented in table 4-14.

Dose Groups Statistically Significantly Equal to the Control		
	MALE	FEMALE
T3	.11 mg/kg/day	None
TSH	.44 mg/kg/day	.124 mg/kg/day

Table 4-14. Summary of NOAELs for T3 and TSH.

A summary of the results, conclusions and recommendations are presented in chapter 5.

V. Conclusions and Recommendations

Overview

The primary objective of this research was to determine toxicity information to establish permissible exposure levels of ammonium perchlorate (AP). Sprague Dawley rats were exposed to varying concentrations of AP in their drinking water for a two-week period. The rats were then sacrificed and their hormone levels were measured via radioimmunoassay. The goal of the statistical analysis was to determine a threshold dose based on the hormone level data. The sigmoidal function did not accurately characterize the data. However, the NOAEL for AP on TSH levels was 0.443 mg/kg/day for the male rats and 0.124 mg/kg/day for the female rats.

Summary of Findings

Average water consumption

AP did not have a statistically significant affect on the average water consumption of either sex at the concentrations administered.

Body weight gain

AP did not have a statistically significant affect on the body weight gain of either sex. Both sexes gained weight in the same manner over the two-week period.

Thyroid/Body weight ratio

AP had a statistically significant affect on the thyroid/body weight ratios of the rats exposed 11.4 mg/kg/day and higher. The thyroid/body weights in these dose groups experienced an increase in thyroid body weight ratios as compared with the control group.

Thyroid hormone levels

AP had a statistically significant affect on the thyroid hormone levels in both sexes and the sexes were not affected in the same way. Triiodothyronine (T3) levels in male and female rats fell, Thyroxine (T4) levels remained relatively unchanged. Reverse Triiodothyronine (rT3), Thyrotropin (TSH), and Thyroglobulin (Tg) all increased in both males and females.

Data

The data derived from the two-week study could not be used to establish a dose-response function in order to extrapolate the dose level which corresponds to two standard deviations above the mean hormone level for the control group. It appeared that the dose range was not optimum and that lower doses were needed. However, a Tukey comparison of means revealed a NOAEL of .44 mg/kg/day for the male rats and .124 mg/kg/day for the female rats. These results are consistent with the assumption made by Dollarhide (1992) that .14 mg/kg/day was the NOAEL for perchlorate. Based on these NOAELs a RfD of 4.133×10^{-4} is recommended. This reference dose proposes an uncertainty factor of 300. Ten for the use of less than a chronic study, ten for the protection of sensitive individuals and three for the application of animal data to humans.

Since Capen (1992) has shown that the rat thyroid is more sensitive than the human thyroid, the later uncertainty factor was halved on a log scale to 3.

Future Research

Although there was enough data to establish a NOAEL for AP, more research is needed in order to determine the estimated threshold dose. Future studies should establish dose ranges around an estimated threshold between .124 mg/kg/day and .46 mg/kg/day. In addition, the NOAEL should not be divided by overly conservative safety factors in light of this study's NOAEL which confirms the range assumed by the EPA and because it is now known that the rat thyroid is more sensitive than the human (Capen, 1992).

NOTE: This assessment was prepared for the sponsors by an independent party. It has been included in this package to demonstrate alternative approaches that have been taken in the development of a RfD for perchlorate. The RfD in this report is not subject to review at this meeting; it has been included for informational purposes only. *TERA* does not necessarily agree or disagree with the conclusions of this report.

DRAFT

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TECHNICAL MEMORANDUM, DETERMINATION OF RISK-BASED SAFE LEVEL FOR PERCHLORATE IN GROUNDWATER, GENCORP-AEROJET PROPULSION DIVISION SACRAMENTO, CALIFORNIA

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JANUARY 1996

TABLE OF CONTENTS

EXECUTIVE SUMMARY

This document establishes 4 mg/L as a risk-based safe level for perchlorate in groundwater at the Aerojet site in north central California. This risk-based safe level is based upon the assumption that a resident drinks two liters and bathes in this water daily. The perchlorate anion (ClO_4^-) exists on the Aerojet site mostly in the form of ammonium perchlorate (NH_4ClO_4), although 4 mg/L is also safe for other perchlorate salts.

When consumed at high doses for prolonged periods, perchlorate exacerbates the frequency of thyroid cancer. However, its mechanism of carcinogenesis is clearly understood and requires the growth of the thyroid gland into a goiter as an intermediate step. Below the goitrogenic dose there are no carcinogenic risks from perchlorate exposure.

A reference dose (RfD) of 0.12 mg/kg/day has been calculated for perchlorate on the basis of noncarcinogenic adverse health effects, and this RfD is used to derive the risk-based safe level of 4 mg/L in groundwater as presented in this report. The RfD is derived from the plethora of human data from Graves' disease patients treated with high doses of perchlorate as well as toxicologic studies of animals treated with lower doses of perchlorate. It allows for incorporation of a 100-fold safety factor in determination of a safe dose.

For determination of 4 mg/L as a safe level of perchlorate in groundwater at the Aerojet site, two exposure pathways have been considered:

- Ingestion of groundwater at two liters per day; and
- Dermal contact with groundwater during bathing.

Since perchlorate penetrates skin poorly, the latter pathway contributes insignificantly to total potential risk. Groundwater ingestion predominates as the major pathway of significance. The safe level of 4 mg/L in groundwater is ten times the 0.4 mg/L detection limit for perchlorate.

SECTION 1

INTRODUCTION

The purpose of this document is to establish a safe level of perchlorate in groundwater. Following a discussion of toxic mechanisms, a safe level of perchlorate in groundwater is derived based upon its calculated reference dose and potential human exposures through ingestion and dermal contact. Appendix A of this document explores the toxicology of perchlorate more completely and presents a glossary of technical terms. A complete list of references may be found at the end of this report.

Perchlorate has been used in the past as an oxidizer in rocket propellants, and residual concentrations are found in groundwater at the Aerojet site. Although perchlorate has been utilized medicinally to treat hyperthyroid patients, there is not an extensive environmental reference base for perchlorate toxicity. Since it has been shown to increase thyroid cancer frequency when fed to test animals at high doses and since it is highly water soluble, there has been some concern as to what constitutes a safe level in groundwater. Present analytical techniques are not capable of measuring perchlorate below 0.4 mg/L in water samples.

The reason that perchlorate (ClO_4^-) is useful in rocket fuel is that it is very active as a oxidizer (i.e., it gives up the 4th oxygen quite readily). If reducing agents are encountered in the environment, perchlorate is readily reduced to chlorate (ClO_3^-). As will be shown, all perchlorate toxicities are mediated by its action on the thyroid. Since the chlorate anion has no effect upon the thyroid gland, it is not toxic. Perchlorate salts are freely water-soluble and dissociate such that the toxicologies of various salts (NH_4, Na, K, etc.) are very similar.

SECTION 2

TOXICOLOGY OF PERCHLORATE (OVERVIEW)

The perchlorate anion (ClO_4^-) blocks iodine uptake through competitive binding with the iodide transporter. When consumed at high doses for prolonged periods, perchlorate exacerbates the frequency of thyroid cancer. However, its carcinogenic mechanism is clearly understood and requires prolonged hyperthyroid growth (goitrogenesis). There is no carcinogenic risk if exposures to perchlorate are below the goitrogenic threshold dose.
← which is?
Therefore, a human reference dose (RfD) of 0.12 mg/kg/day has been derived based upon noncarcinogenic toxicities of perchlorate and the application of appropriate safety factors. The 0.12 mg/kg/day RfD for perchlorate is utilized herein to derive a safe residual level in groundwater based on ingestion and dermal contact. Specific calculations for each route of exposure are presented in Section 3; Section 4 summarizes the results of the risk calculations.

The available epidemiologic and experimental data in support of this RfD for perchlorate are detailed and referenced in Appendix A of this report. These toxicological data for perchlorate include interspecies comparisons, sexual differences, genotoxic, developmental and reproductive data, as well as a discussion of potential target organ sites other than the thyroid gland. A glossary of technical terms is provided at the end of this report.

Available toxicological data for perchlorate is very consistent based on several human studies and animal experiments. The highest No Observed Adverse Effect Level (NOAEL) and lowest Lowest Observed Adverse Effect Level (LOAEL) data in humans, rats and other animals are within an order of magnitude, with 12 mg/kg/day representing a "NOAEL/LOAEL interface" in healthy human volunteers.

Males and females have equivalent sensitivities to perchlorate toxicity. Only the thyroid gland is adversely affected by perchlorate doses which are below gram/day levels in humans. The NOAEL for reproductive and developmental effects, including fetotoxicity, is also well above the threshold for perturbation of the thyroid-pituitary axis.

As defined by USEPA, data for the determination of reference doses (RfDs) are best based upon the highest NOAEL available from chronic studies in humans. Prior to Brabant's recent studies (Brabant et al 1992,

1994, 1995), the best available human data were from Stanbury and Wyngaarden (1952). However, there are several advantages to the more recent Brabant studies:

- Brabant's subjects were normal healthy individuals, whereas Stanbury and Wyngaarden's subject was hyperthyroid;
- Brabant used 10 subjects, whereas Stanbury and Wyngaarden studied but one subject; and
- Brabant's studies were subchronic, whereas Stanbury and Wyngaarden's study was acute.

As explained further in Appendix A, due to further experimentation in 1995, the Brabant et al (1994) NOAEL of 12 mg/kg/day is better interpreted as a NOAEL/LOAEL interface, in that thyroid volumes increased slightly but significantly during the fifth week of daily administration of perchlorate at 12 mg/kg/day. Neither decreased T3/T4 nor increased TSH levels were observed in individuals who experienced these slight increases in thyroid volume. Hench, 12 mg/kg/day is a NOAEL for the thyroid-pituitary axis as normally defined, but a slight LOAEL for thyroid volume. For the reasons stated above, this endpoint would appear superior for RfD determination than the formerly utilized LOAEL of 1.4 mg/kg/day from the study of Stanbury and Wyngaarden (1952).

Thus, it is recommended that the NOAEL/LOAEL value of 12 mg/kg/day perchlorate be adopted as the basis for a reference dose with application of two tenfold safety factors for subchronic-to-chronic extrapolation and protection of potentially sensitive populations. Among the potentially sensitive populations covered by incorporation of this second tenfold safety factor are children. Thus, the reference dose derived in this analysis is 0.12 mg/kg/day (i.e., 12 mg/kg/day ÷ 100). The threshold dose required for perchlorate-induced thyroid carcinogenesis is above the 12 mg/kg/day dose which has been used to establish this RfD. Hence, the question of carcinogenicity by perchlorate is moot, since the reference dose chosen is protective of this as well as other thyroid-mediated effects.

SECTION 3

RISK ASSESSMENT FOR PERCHLORATE IN GROUNDWATER BY EXPOSURE ROUTE

Potential risks to adults from exposure to 4 mg/L perchlorate in groundwater are calculated for two routes of exposure: ingestion and dermal contact. Evaluation of inhalation exposure to perchlorate in ground water is not required since perchlorate is not volatile. Default assumptions for exposure amounts via each route are discussed below and annotated. These default parameters are based on an unrestricted use scenario (i.e., that groundwater is used as drinking water for residential housing or other similar use). For example, it is assumed that adults spend their entire day at the site, which is overly conservative for the adult on-site worker. Risk is defined as the hazard index, (i.e., the ratio of dose via each exposure pathway to a safe reference dose). If the total hazard index by both exposure routes exceeds one, adverse health effects may occur as a result of the defined conditions of exposure.

Ingestion

There are many default values for groundwater ingestion. Ingestion of two liters of water per day (2L/day) has been used routinely as a default value for adults (USEPA, 1989; California, 1994), although other default values may be used for children (USEPA, 1989; California, 1994). Use of these factors (i.e., ingestion of 2 liters of water a day by a 70 kg adult) is consistent with the approaches used by USEPA to derive drinking water health advisories and to evaluate maximum contaminant levels (MCLs), and is consistent with USEPA Region IX's calculation of tap water preliminary remediation goals (PRGs). Default ingestion scenarios assume total groundwater consumption, (i.e., in coffee and other products containing groundwater as well as for drinking water itself).

Using the default exposure assumptions developed by the California Department of Toxic Substances Control (California, 1994) for an adult under a residential scenario, the daily ingestion dose is calculated as follows:

$$\frac{2\text{L/day} \times 4\text{ mg/L} \times 350\text{ days/year} \times 24\text{ years}}{70\text{ kg} \times 24\text{ years} \times 365\text{ days/year}} = 0.11\text{ mg/kg/day}$$

Since the reference dose for perchlorate is 0.12 mg/kg/day, the following hazard index may be obtained:

0.11 mg/kg/day ÷ 0.12 mg/kg/day = 0.913

Thus, for ingestion of groundwater, the proposed 4 mg/L level of perchlorate results in a hazard index that is less than one. Hence, the concentration is considered safe for an adult exposed to this concentration via ingestion of groundwater under an unrestricted use scenario.

Dermal Absorption

Dermal absorption of contaminants in groundwater depends upon multiple physical and chemical properties of the contaminant (USEPA, 1992). Important parameters which determine the extent of dermal absorption of substances in groundwater include: size and molecular weight, water solubility, permeability coefficient in aqueous media (K_p), and octanol/water partitioning coefficient (K_{ow}).

Given that site groundwater is within the normal range of physical and chemical parameters, the most important drivers of dermal absorption are the combination of solubilities of a given contaminant in water and organic solvents. Those contaminants which have infinite water and organic solvent dissolution, such as dimethyl sulfoxide (DMSO), are absorbed virtually 100 percent by skin. On the other hand, highly polar or highly apolar compounds are poorly absorbed by skin. For example, addition of a nitrogen to the aromatic ring of benzoic acid to form nicotinic acid increases polarity, renders it lipid insoluble and greatly delimits dermal absorption (USEPA, 1992). Inorganic ions are more highly polarized than nicotinic acid, and have limited solubilities in organic solvents such as ether and ethanol; therefore, their dermal absorptions are normally less than 10 percent and frequently less than 1 percent.

Perchlorates (ClO_4^-) are freely soluble in water, but are insoluble in ether and have limited solubilities in ethanol and methanol (Merck, 1983). Water solubilities for four perchlorate salts at 25°C are within the range of 20-80 percent (CRC, 1996), as summarized below.

Perchlorate Water Solubilities
(CRC, 1996)

Perchlorate	Water Solubility (%)
Ammonium (NH_4ClO_4)	19.7
Lead ($Pb[ClO_4]_2$)	81.5
Nickel ($Ni[ClO_4]_2$)	52.8
Sodium ($NaClO_4$)	67.2

Although no published K_p value for any perchlorate could be found, it is probable that cadmium chloride ($CdCl_2$) is a reasonable surrogate. Like perchlorates, cadmium chloride is freely soluble in water (54.6 percent at 25°C), insoluble in ether and slightly soluble in ethanol and methanol. The permeability coefficient of cadmium chloride is 0.0012 cm/hr (USEPA, 1992).

Given that various perchlorate salts, including ammonium perchlorate, have relevant physical and chemical characteristics that are similar to cadmium chloride, cadmium chloride represents a reasonable surrogate value for perchlorate. This approach is consistent with the methodology recommended by USEPA (1992) for evaluation of inorganic constituents in water.

If one assumes that an adult is dermally exposed through bathing to 4 mg/L perchlorate in groundwater at the Aerojet site, the following equation pertains (USEPA, 1989; California, 1994):

$$DAD = \frac{GC \times CF \times SA \times K_p \times T \times EF \times ED}{BW \times ED \times 365\ days/year}$$

Where:

DAD = Dermally absorbed dose (mg/kg/day)
GC = Groundwater concentration (4 mg/L)
CF = Conversion factor (10^{-3} L/cm^3)
SA = Skin surface (23,000 cm^2, adult; California, 1994)
K_p = 0.0012 cm/hr
T = 0.14 hrs/day (California, 1994)
EF = Exposure frequency (350 days/year; California, 1994)
ED = Exposure duration (24 years, adult; California, 1994)
BW = Body weight (70 kg, adult; California, 1994)

Thus, for an adult, the following dose may be calculated:

$$DAD = \frac{4 \times 10^{-3} \times 23,000 \times 0.0012 \times 0.14 \times 350 \times 24}{70 \times 24 \times 365} = 0.00021 \text{ mg/kg/day}$$

Since the reference dose for perchlorate is 0.12 mg/kg/day, the following hazard index may be obtained:

$$0.00021 \text{ mg/kg/day} \div 0.12 \text{ mg/kg/day} = 0.002$$

Thus, the proposed 4 mg/L level of perchlorate in groundwater results in a hazard index that is well below one for an adult receptor exposed via dermal contact under a residential scenario, and no adverse health effects would be anticipated as a result of the defined exposure.

SECTION 4

CONCLUSION

As detailed in Appendix A, a reference dose (RfD) of 0.12 mg/kg/day has been derived for perchlorate. This RfD has been utilized in the previous section to calculate potential risk from 4 mg/L perchlorate in groundwater for an adult by ingestion and dermal exposure under an unrestricted land use scenario. The foregoing calculations yielded the following hazard indices:

Exposure Route	Hazard Index
Ingestion	0.913
Dermal contact	0.002
TOTAL	0.915

As shown above, the maximum total hazard index (0.915) is less than one. Four mg/L perchlorate was derived as the concentration in groundwater that would present no risk by ingestion and dermal contact based upon the reference dose of 0.12 mg/kg/day. It should also be noted that only if individuals drank and bathed in water containing 100 times this concentration (400 mg/L perchlorate) each day for over one month would the first signs of thyroid toxicity appear (Brabant, 1994).

SECTION 5

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APPENDIX A

DETAILED TOXICOLOGICAL PROFILE FOR PERCHLORATE

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SECTION A1

INTRODUCTION

Overview of Perchlorate Toxicity

Perchlorate, ClO_4^-, is an anion which forms salts with most cations. Monovalent cation salts of sodium ($NaClO_4$), potassium ($KClO_4$) and ammonium (NH_4ClO_4) perchlorate have found wide use as rocket propellants, ignitable sources and, medicinally, for control of hyperthyroidism. Perchlorate is still used today for the control of hyperthyroidism in Germany. Since perchlorate salts all dissociate completely when dissolved in water or aqueous tissues, their toxicities are equivalent (although doses must be adjusted slightly to account for molecular weight differences of the cations). As will be annotated further below, the sole toxicologic mechanism of perchlorate in the 1-10 mg/kg/day dose range in a variety of mammalian systems is to block iodine uptake by the thyroid gland.

USEPA has adequately dismissed the issue of thyroid carcinogenicity by perchlorate (USEPA, 1988; Hill et al, 1989; USEPA Risk Assessment Forum, *in press*). Perchlorate is nongenotoxic and its mechanism for thyroid carcinogenesis requires long-term exposure to very high concentrations. The USEPA has concluded that perchlorate and other thyroid carcinogens have carcinogenic threshold concentrations.

As spelled out further below, perchlorate, like many other thyroid carcinogens, acts by blocking iodine uptake and inducing goitrogenesis via chronic exposure. Similarly, a mere lack of iodine in the diet increases risk for thyroid cancer also mediated through goitrogenesis. The fact that the carcinogenicity of perchlorate may be mimicked by iodine deprivation not only defines its mechanism of action but also indicates a dose-response relationship which contains a threshold (i.e., that perchlorate dose which blocks iodine uptake sufficiently to cause goiter formation). The mechanism for thyroid carcinogenesis by those agents which block iodine uptake has been recently reviewed (Thomas, 1994).

Since the threshold dose for perchlorate induction of goiter formation is quite high, other lower toxic doses are more useful in setting safe levels based upon noncarcinogenic activities. However, it should be noted that

virtually all toxicities associated with perchlorate are mediated by its adverse effect on hormone synthesis by the thyroid gland.

Perchlorate Carcinogenicity and Goitrogenesis

The USEPA Risk Assessment Forum has discussed overall mechanisms of thyroid follicular cell carcinogenesis by goitrogens which block iodine uptake (USEPA, 1988; Hill et al, 1989; USEPA *in press*). Experimental perchlorate thyroid carcinogenesis requires goitrogenesis and, hence, is mediated by the same iodine-blocking mechanisms which cause depression of triiodothyronine (T3) and thyroxin (T4) along with elevation of thyroid stimulating hormone (TSH). These are the cardinal signs of disturbance of the thyroid-pituitary axis. If the thyroid-pituitary axis is not disturbed, there is no carcinogenic risk. Animals treated with perchlorate at carcinogenic levels are prevented from thyroid carcinogenesis if given exogenous T3/T4 (Paynter et al, 1988). Hence, the threshold concentration of perchlorate below which there is no depression of T3/T4 with TSH elevation is completely protective for carcinogenesis.

Both the Paynter et al (1988) and Hill et al (1989) papers represent USEPA's endorsement of a threshold mechanism for thyroid follicular carcinogenesis which depends upon goitrogenesis resulting from derangement of the thyroid-pituitary axis, (i.e., depressed T3/T4 with elevated TSH). For these reasons, despite USEPA's classification of B2 carcinogenicity, there is no carcinogenic risk from perchlorate at levels below the NOAEL for disruption of the thyroid-pituitary axis. Hence, carcinogenesis as an endpoint will not be discussed further in this paper and the discussion instead will focus on setting a reference dose (RfD) for noncarcinogenic effects.

NOAEL/LOAEL Values for Noncarcinogenic Effects

Reference doses (RfDs) for the protection of human health are optimally derived from human data, if available. Because of its widespread use in the chemotherapy of hyperthyroidism, as much human data exists for perchlorate as for any other potentially toxic substance. For other less well studied toxicants, precise dosimetry is derived from animal experiments and adjusted for human risks via application of safety factors (Dourson, 1994). Critical values in the determination of safe doses are the NOAEL (No Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level), the highest dose showing no adverse health effect and the lowest dose showing such an effect, respectively.

A careful compilation of available studies of the perchlorate toxicity database shows a consistency of effects across species and sensitive individuals with NOAEL and LOAEL values agreeing within the same order of magnitude. Hence, it is recommended that no safety factors should be used for database insufficiency or for interspecies extrapolation (see further discussion in Section 5).

SECTION A2

STUDIES OF PERCHLORATE TOXICITY IN HUMANS

Most studies of perchlorate in humans and experimental animals have utilized doses ≥5 mg/kg/day; fewer contain the low dose information relevant to the establishment of a dose-response relationship between perchlorate exposure and disturbance of the thyroid-pituitary axis, which is the most sensitive measure of perchlorate toxicity. Much of the human data concerning perchlorate toxicity come from case studies of hyperthyroid patients treated with perchlorate to reduce thyroid volume. There are less data concerning NOAEL and LOAEL values in healthy humans. The one epidemiologic study of perchlorate workers could not discern the effects of perchlorate itself, since many chemical exposures existed simultaneously (Rockette and Arena, 1983).

Studies in Normal Human Volunteers

Three studies of normal human volunteers treated with perchlorate exist. They are Brabant et al (1992, 1994, 1995), Burgi et al (1974) and Shigan (1963). These studies are summarized below.

Brabant et al (1992, 1994, 1995)

Perchlorate continues to be utilized in Germany for the control of hyperthyroidism. Dr. Georg Brabant, a clinical endocrinologist at the Medizinische Hochschule in Hanover, has been conducting research into the mechanisms of perchlorate action for the past five years. In the Brabant et al (1992) study, five healthy male volunteers in their mid-20s were treated for four weeks with 200 µg/day iodine followed by 900 mg/day (12 mg/kg/day) perchlorate for an additional four weeks. T3/T4 and TSH levels were followed during a 24 hours period at the end of both iodine and perchlorate treatments. T3/T4 levels were not altered by perchlorate treatment and TSH levels *decreased* slightly during the four weeks on perchlorate. Hence, from the published Brabant et al (1992) study, a human NOAEL for perchlorate for perturbation of the thyroid-pituitary axis appears to be 12 mg/kg/day.

Brabant et al satisfied the above former technical definition of a NOAEL for perchlorate induction of hypothyroidism (lack of T3/T4 depression, no elevation of TSH). In further experiments with healthy male volunteers, Brabant et al (personal communication, 1994, 1995) have shown that treatment with 12 mg/kg/day perchlorate for longer than four weeks results in a slight, but statistically significant increase in thyroid volume for all treated human subjects, even though TSH is never seen to increase. Their interpretation is that enhanced thyrocyte sensitivity to TSH is an adaptive response which is as important as increased TSH levels in the human response to inhibited iodine uptake. Hence, 12 mg/kg/day may be interpreted as a human NOAEL/LOAEL, (i.e., at the interface between NOAEL and LOAEL values), rather than as a strict NOAEL *per se*.

Dr. Brabant is in the process of writing up his latest research for publication in a peer-reviewed journal (Brabant, *in press*). Within the past year his group has realized that subtle, diurnally adjusted changes in thyroglobulin levels may be the most sensitive index of perchlorate activity. It is anticipated that this endpoint may allow precise titration of a human NOAEL. In addition, longer term followup of his volunteer cohort after cessation of perchlorate treatment has shown that slight increases in thyroid volumes quickly return to normal.

Burgi et al (1974)

Burgi et al (1974) examined the effect of 200 mg perchlorate administered three times daily for one week to five healthy volunteers on the fate of radioiodines administered 17 days (as iodine-125) and 6 days (as iodine-131-thyroxin) previously. Average weight of these five volunteers was 61.8 kg. Burgi et al's complicated protocol was designed to determine if perchlorate could displace all incorporated radioiodines from the human thyroid gland. Since part of the endogenous radioiodine was purged from thyroid glands in this study, 9.7 mg/kg/day is calculated as a LOAEL sufficient for disturbance of the thyroid-pituitary axis.

Shigan (1963)

Shigan (1963) utilized urinary excretion of administered iodine-131 as a measure of iodine uptake in normal human healthy volunteers fed ammonium perchlorate. Four of five volunteers had increased urinary excretion of iodine-131 following ingestion of 2.9 mg/kg/day, which is a LOAEL in this study. Further details concerning this Russian study are not available, but these human data compared favorably with experimental data from rats reported in the same paper (see discussion below).

Studies in Hyperthyroid Patients

Although case studies are somewhat useful in the absence of valid epidemiologic data, they are limited in their statistical power. For all but one patient, who was exposed to perchlorate for only a few hours, treatment was prolonged at very high doses of perchlorate and determination of NOAEL or LOAEL levels is precluded. The one exception comes from the short-term study of Stanbury & Wyngaarden (1952) in which one hyperthyroid patient showed a LOAEL of 1.4 mg/kg/day and a NOAEL of 0.14 mg/kg/day.

Chronic treatment of Graves' disease patients with high levels of perchlorate has resulted in agranulocytosis and aplastic anemia. It is most likely that secondary chronic effects of perchlorate administration are mediated by loss of T3/T4 and the hormonal effects of subnormal triiodothyronine and thyroxin levels on bone marrow production of blood cells. Higher levels of perchlorate are required for methemoglobulinemia and irritation of oral or gastric mucosa, eyes or skin. Hence, overall, levels that are protective for thyroid-pituitary axis dysfunction (i.e., those endpoints utilized for NOAEL/LOAEL/RfD calculations), are protective for all known adverse effects of perchlorate.

In patients treated with perchlorate at >15 mg/kg/day for Graves' disease, some skin rashes (6/240), nausea (5/240) and agranulocytosis (1/240) were seen (Crooks and Wayne, 1960). Additional cases of agranulocytosis were also described by Barzilai and Sheinfeld (1966), Southwell and Randall (1960) and Sunar (1963). In Morgans and Trotter (1960), 3% of 180 patients treated with ≥6 mg/kg/day and 18% of 67 patients treated with ≥20 mg/kg/day perchlorate developed skin rashes, sore throats and gastrointestinal irritation. One fatal acute liver atrophy developed in a patient treated with 10 mg/kg/day perchlorate for 13 months (Kotzaurek, 1965). Another case report describes nephrotic syndrome in a patient treated with 11 mg/kg/day for 5 months (Weber and Wolf, 1969). In the 1960s, several patients receiving ≥10 mg/kg/day perchlorate for Graves' disease therapy developed fatal aplastic anemias (Hobson, 1961; Johnson and Moore, 1961; Fawcett and Clarke, 1961; Krevans et al, 1962; Gjemdal, 1963; Barzilai and Sheinfeld, 1966). Although these side effects of prolonged perchlorate treatment are serious, two constraints on the interpretation for setting a reference dose must be noted:

- None of the observed side effects occurred in patients who received less than 6 mg/kg/day perchlorate.

- Similar side effects have been noted in other therapeutic regimes for hyperthyroidism, including propylthiouracil and carbimazole (Everd, 1976; Biswas et al, 1991). Use of these chemotherapeutic regimes also required high doses for elicitation of adverse effects.

groups. The dams which had not given birth in both groups showed no signs of implantation.

In a subsequent study, Brown-Grant and Sherwood (1971) also fed potassium perchlorate at 1 percent in drinking water to gravid Wistar rats, but in this case the rats were lactating, to delay implantation, and 0.1 percent potassium iodide served as the control. The feeding scheme was also prolonged from the day of conception to the 12th or 13th day. Again, there was not a significant effect of perchlorate on implantation. When pup thyroids were examined, a significant increase in weights of the perchlorate-treated group was noted (~50 percent), which is similar to the adult response at this high dose. Although perchlorate can cross the placenta, it does not affect blastocyst survival in the rat. It is concluded that at 100 times the NOAEL, perchlorate crosses the placenta where its only effect is to enlarge the fetal thyroid gland. Perchlorate is not a reproductive or developmental toxin at this very high dose.

Genotoxicity data

A thorough review of all available scientific literature reveals no tests for genotoxicity of perchlorate. Chemically, there is no reason to suspect that perchlorate would react with DNA or that its presence in cells would prove disruptive to chromosomal structure or replication. The mechanism by which perchlorate is carcinogenic for the thyroid gland at high concentrations requires blocked iodine uptake and subsequent goitrogenesis and does not depend upon any genotoxic activity. Given the known mechanism of goitrogenesis and lack of data for mutagenicity or clastogenicity, it is concluded that perchlorate is not genotoxic.

SECTION A4

COMPILATION OF STUDIES USED FOR ESTABLISHING A REFERENCE DOSE

A plethora of data exists for high doses of perchlorate administered to hyperthyroid patients. Although relatively few normal human and experimental studies of perchlorate toxicity are available from which to evaluate dosimetry, the agreement between all these various studies is striking. These data are compared in the following subsections.

Human NOAEL and LOAEL Values

Three studies have been conducted in normal human volunteers administered perchlorate for various periods of time. In the first Brabant study (Brabant, 1992), as measured by T3/T4 diminution and TSH stimulation, there were no effects in five healthy volunteers given 12 mg/kg/day after four weeks. However, in a followup study utilizing another five healthy volunteers, although the T3/T4/TSH levels were not perturbed, some increase in thyroid volume occurred after administration of 12 mg/kg/day beyond the fourth week. These data along with those of Burgi et al (1974) and Shigan (1963) are summarized in the following table.

TABLE 2

Comparison of NOAEL and LOAEL Values in Human Studies

Reference	NOAEL	LOAEL	Effect
Brabant et al (1992)	12 mg/kg/d		T3/T4 not decreased TSH not increased
Brabant et al (1994)		12 mg/kg/d	Thyroid volume increase
Burgi et al (1974)		9.7 mg/kg/d	Depletion of I-131 from thyroid
Shigan (1963)		2.9 mg/kg/d	Depletion of I-131 from thyroid

SECTION A3

STUDIES OF PERCHLORATE TOXICITY IN ANIMALS

In general, perchlorate toxicities in domestic or experimental animals mirror those seen in human volunteers or patients receiving perchlorate therapeutically. Disruption of the thyroid-pituitary axis is the main mammalian toxicity of perchlorate and a threshold for this effect is seen in animals as well as in humans. As a beneficial effector, perchlorate salts have been utilized in Russia for increasing the weight of domestic animals (Yakimenko et al, 1981). Weight gains of up to 31 percent as compared to controls were seen with 5 mg/kg/day ammonium perchlorate, comparable to the LOAEL values seen in other studies.

Acute Studies in Experimental Animals

There are many reported acute toxicity experiments of perchlorate administration to animals. Perchlorate is moderately toxic to rats, with the LD_{50} in the range of 1-4 g/kg, (Praeger and Sax, 1982). However, acute toxicity experiments are of little value in establishing a reference dose and, therefore, are not considered further here.

Chronic Studies in Experimental Animals

The only chronic studies of perchlorate toxicity in experimental animals have to do with carcinogenesis and, as discussed above, are not directly relevant to establishment of a reference dose. Studies are summarized below which clearly demonstrate that perchlorate is not an initiator of thyroid carcinogenesis.

Hiasa et al (1987) fed male Wistar rats 0 or 1000 ppm perchlorate for 20 weeks and measured T3, T4 and TSH levels, as well as body and liver weights and appearance of thyroid tumors. Although TSH levels increased, T3 and T4 changes were not statistically significant. No thyroid tumors were seen. In separate groups of 20 rats first injected with 28 mg/kg n-bis(2-hydroxypropyl)nitrosamine and then fed perchlorate, 20/20 developed thyroid tumors, whereas without perchlorate treatment only 1/20 developed tumors. There were no effects of perchlorate on

body or liver weights. Exactly the same promotion of initiated thyroid tumors may be mediated by iodine deficient diets alone, suggesting that this is the sole activity of perchlorate (Ohshima and Ward, 1986).

The experimental studies of perchlorate dose-response for perturbation of the thyroid-pituitary axis and mechanism of carcinogenesis are prototypic in demonstrating a threshold for carcinogenic promotion. The evidence for thyroid tumor promoter thresholds has been summarized by USEPA (USEPA, 1988; Paynter et al, 1988; Hill et al, 1989) and the implications for risk assessment of perchlorate and other nongenotoxic thyroid tumor promoters reviewed recently by McClain (1992).

Subchronic Studies in Experimental Animals

Whereas most laboratory studies of perchlorate carcinogenicity using experimental rodents have been chronic, most dose-response studies have been subchronic. Of particular value is Männistö et al (1979), a four day study in rats which received four concentrations of perchlorate in drinking water (the concentrations embraced NOAEL and LOAEL doses for perturbation of the thyroid-pituitary axis).

The data of Männistö et al (1979) are useful in that a wide range of perchlorate doses were administered. These data are summarized below.

TABLE 1

T3/T4 and TSH Levels in Perchlorate-Treated Rats (Männistö et al, 1979)

Perchlorate (mg/L)	mg/kg/day	T3/T4	TSH
0	0	No change	No change
10	1.5	No change	No change
50	7.6	Decrease	Slight increase*
100	15.3	Decrease	Increase
500	76.3	Decrease	Increase

*The slight increase in TSH was not statistically significant.

Given the definition of perturbation of the thyroid-pituitary axis (i.e., T3/T4 levels must be depressed while TSH is elevated, both in statistically significant manners), it is concluded from the Männistö et al (1979) data that 7.6 mg/kg/day is a NOAEL and 15.3 mg/kg/day is a LOAEL for perchlorate in the rat.

Comparison of the Männistö et al (1979) and Brabant et al (1992) LOAEL data challenges the assumption that rats have a different sensitivity to perchlorate than humans. Whereas rats showed a slight, statistically significant, increase in TSH at 15.3 mg/kg/day perchlorate, TSH remained depressed at 12 mg/kg/day in human volunteers, although thyroid volume increased slightly after four weeks (Brabant et al, 1994). Hence, the LOAELs derived from these studies are similar: 15.3 and 12 mg/kg/day, in the rat and in humans, respectively.

Kessler and Kruskemper (1966) fed 1 percent potassium perchlorate (~1300 mg/kg/day) to 40 rats maintained with 40 controls. Groups of 6-8 rats were sacrificed immediately and after 40, 120, 220 and 730 days of treatment. There was no influence of 1,300 mg/kg/day on body weight. Thyroid glands, however, were hypertrophied, with histological changes being detected by 40 days and progressing throughout the experiment through fibrosis and on to follicular adenomas. Gauss (1972) conducted similar experiments in mice, with similar effects seen on the thyroid gland, but also minor weight loss (11.6 percent) during the first two months of treatment (as cited in ECAO, 1992).

To study iodine uptake by the rat and rabbit thyroid, Shigan (1963) used iodine-131, which was administered a day following ingestion of perchlorate; test doses of perchlorate were 0, 0.25, 2 and 40 mg/kg/day. Urinary excretion of iodine-131 was higher than controls in the 2 and 40 mg/kg/day groups, although not higher at 40 than 2 mg/kg/day. By this criterion, 0.25 mg/kg/day perchlorate did not block iodine-131 uptake and, hence, was the NOAEL in this study, while 2.0 mg/kg/day was the LOAEL.

Other Organ Site Studies in Experimental Animals

Pflugfelder (1959) studied the effect of ingested potassium perchlorate on the thyroid and other organs of the chicken. Daily doses were 20, 30 and 40 mg/kg to groups of three chickens for each dose. Although it appears as if this was a chronic study, it is not clear how long chickens were maintained on these perchlorate doses prior to sacrifice and necropsy. Thyroid volumes were reduced at all doses as was body weight gain. Other organ toxicities noted at all doses included: Failure of the bursa of fabricius (an organ for the maturation of B-lymphocytes), diminished feather exfoliation, lessened sexual development and degeneration of cerebellar Purkinje cells. Hence, in the chicken, it appears that 20 mg/kg/day perchlorate is a LOAEL for several target organ sites, albeit with limited significance for assessing human risks.

Sreebny et al (1963) studied the effects of drinking water with 1 percent $KClO_4$ to male Sprague-Dawley rats for 30 or 60 days on three exocrine glands: The submaxillary gland, parotid gland and pancreas. Although submaxillary gland and pancreatic weights were reduced as was amylolytic activity of the parotid, these effects were also caused by propylthiouracil and followed induction of thyroid hyperplasia in both cases. Hence, the observed effects on exocrine glands in this experiment were indirectly mediated by activity of perchlorate on the thyroid gland.

Hiasa et al (1987) found no effects on liver or body weights from feeding 1000 ppm perchlorate to male rats for 20 weeks.

Shigan (1963) studied effects of perchlorate in rats and rabbits dosed with 0.25, 2 and 40 mg/kg/day orally. Several measured endpoints were negative at all doses:

- Involuntary regulation of cardiac activity,
- Central nervous system functions,
- Hemoglobin synthesis,
- Protein synthesis, and
- Liver function.

Hence, for organs other than the thyroid, the NOAEL for perchlorate in rats and rabbits is in excess of 40 mg/kg/day.

Reproductive, Developmental and Genotoxicity Studies

Reproductive and developmental toxicity studies

Postel (1957) fed pregnant guinea pigs with 1 percent potassium perchlorate (740 mg/kg/day) in drinking water from the 21st through the 48th day of gestation and noted a 15-fold enlargement of fetal thyroids. This fetotoxic dose is 100 times the NOAEL seen in other experimental studies. The same treatment regimen was too short to enlarge adult guinea pig thyroids, which required 60 or more days of exposure to perchlorate for thyroid enlargement.

Brown-Grant (1966) also fed potassium perchlorate at 1 percent in drinking water (740 mg/kg/day) to gravid Wistar rats from the 2nd to the 8th day of gestation. One percent KCl served as the control dose. Live litter births occurred in 8/11 perchlorate-treated and 7/11 KCl-treated

It may be seen that these values are in fair agreement over a four-fold range and, as summarized below, also in fair agreement with data obtained from hyperthyroid patients and in experimental animals.

All volunteers in both Brabant et al (1992, 1994) studies and the Shigan (1963) study were male. However, in the Burgi et al (1974) study, of the five volunteers, two were male and three female. Hence, it is possible from their experimental data to assess the overall sensitivities of human male vs. female thyroids to 200 mg perchlorate being administered three times daily for a week. These data are especially important given the paucity of experimental data in female test animals.

Data from the Burgi et al (1974) study are segregated for normal male and female human subjects and presented in Table 3. Doses of perchlorate , when corrected for body weights, were less in males (8.2 mg/kg/day) than females (11.1 mg/kg/day). No difference was seen between males and females in their urinary excretion of radiolabeled thyroid iodine after treatment with 600 mg perchlorate daily for a week. Males show a 236 percent increase in urinary radioiodine whereas females show a 215 percent increase as measures of mobilization of thyroid iodine by perchlorate. These values are statistically indistinguishable.

TABLE 3

Comparison of Perchlorate Toxicities Between Males and Females (Data taken from Burgi et al, 1974)

		Thyroid Effects**		
Sex (No.)	Dose*	(1)	(2)	(3)
Male (2)	8.2	93	220	236
Female (3)	11.1	101	217	215

*Doses in mg/kg/day for average weight of males at 73 kg and females at 54 kg.
**The following thyroid effects are corrected for relative body weights:
(1) Urinary excretion of radioiodine (µg/day) before perchlorate.
(2) Urinary excretion of radioiodine (µg/day) after perchlorate.
(3) Percent increase in urinary radioiodine after perchlorate treatment.

It is concluded that normal males and females have identical sensitivities to the action of perchlorate on the thyroid gland.

Table 4 compiles LOAEL data from 11 studies of hyperthyroid patients for whom perchlorate was used therapeutically.

TABLE 4

LOAEL Values in Hyperthyroid Patients

Reference	LOAEL (mg/kg/d)
Stanbury & Wyngaarden (1952)	1.4
Crooks & Wayne (1960)	15
Morgans & Trotter (1960)	6
Kotzaurek (1965)	10
Weber & Wolf (1969)	11
Hobson (1961)	
Johnson & Moore (1961)	
Fawcett & Clarke (1961)	All
Krevans et al (1962)	≥10
Gjemdal (1963)	
Barzilai & Sheinfeld (1966)	

It may be seen that there is fair agreement between LOAEL values as determined from case studies and the data discussed previously from normal healthy volunteers.

Experimental Animal NOAEL and LOAEL Values

The results of previously discussed experimental studies are also presented below in tabular form to facilitate comparison of multiple studies on perchlorate toxicity. Following a summary of NOAEL and LOAEL values in experimental animals, these are compared with human values in order to gain an overall appreciation of the general agreement of data across species.

Table 5 summarizes NOAEL and LOAEL values for perchlorate in three animal species for the thyroid and other organ sites.

TABLE 5

Interspecies Comparison of NOAEL and LOAEL Values

Reference	mg/kg/day NOAEL	mg/kg/day LOAEL	Species	Target Organ
Männistö et al (1979)	7.6	15.3	rat	thyroid
Shigan (1963)	0.25	2.0	rat rabbit	thyroid
Shigan (1963)	40		rat rabbit	other organs
Pflugfelder (1959)		2.0	chicken	other organs

As mentioned earlier, virtually all animal studies have employed male test animals, usually male rats, and there are no animal data from which to evaluate male vs. female comparisons. However, some reproductive studies have been done for perchlorate administration to pregnant dams and these results are summarized below.

TABLE 6

Summary of Reproductive Data in Animal Studies

Reference	Dose	Reproductive Effect
Postel (1957)	740 mg/kg/d	Fetal thyroid enlargement
Brown-Grant (1966)	740 mg/kg/d	None
Brown-Grant and Sherwood (1971)	740 mg/kg/d	Fetal thyroid enlargement

Other than the known thyrotoxic effects, there are no reproductive effects of perchlorate administration at very high doses to gravid test animals.

In addition to not being a reproductive toxin, there also are no data to indicate that perchlorate is genotoxic.

The following table summarizes data for target organ sites other than the thyroid gland. Since some organ toxicities depend upon the known

influence of perchlorate on the thyroid and, hence, represent indirect effects, this column is included in the table to facilitate interpretation.

TABLE 7

Summary of Target Organ Data in Animal Studies

Target Organ	Toxicity	Mechanism of Action
Thyroid gland	+	Blocked iodine transport
Liver	-	n/a
Heart	-	n/a
Central nervous system	-	n/a
Bone marrow	+	Thyroxin-dependent*
Submaxillary gland	+	Thyroxin-dependent*
Parotid gland	+	Thyroxin-dependent*
Pancreas	+	Thyroxin-dependent*

*Toxicities which are mediated through impaired T3/T4 synthesis by the thyroid gland.

The three previous discussions are summarized below.

- Males and females have identical sensitivities to perchlorate toxicity;
- Perchlorate is neither a reproductive nor genotoxic substance; and
- The only additional organ sites adversely affected by perchlorate administration besides the thyroid gland itself are those organs whose homeostasis depends upon thyroxin. As indicated in the discussions above, overall there is a surprising concordance of data concerning NOAEL and LOAEL values for the effects of perchlorate on the thyroid gland in normal human subjects, hyperthyroid patients, and experimental animals. These data are summarized in Table 8.

dependent upon thyroxin for normal hematopoietic development (Wartofsky, 1994). Given the consistency of NOAEL and LOAEL values in normal human volunteers and hyperthyroid patients and similar values obtained in experimental animals, it is suggested that the NOAEL/LOAEL of 12 mg/kg/day from Brabant et al (1992, 1994, 1995) be utilized for development of a RfD.

As defined by USEPA, data for the determination of reference doses (RfDs) are best based upon the highest NOAEL available from chronic studies in humans. Prior to Brabant's recent studies (Brabant et al 1992, 1994, 1995), the best available human data were from Stanbury and Wyngaarden (1952). However, there are several advantages to the more recent Brabant studies:

- Brabant's subjects were normal healthy individuals, whereas Stanbury and Wyngaarden's subject was hyperthyroid;
- Brabant used 10 subjects, whereas Stanbury and Wyngaarden studied but one subject; and
- Brabant's studies were subchronic, whereas Stanbury and Wyngaarden's study was acute.

As explained previously, due to further experimentation in 1995, the Brabant et al (1994) NOAEL of 12 mg/kg/day is better interpreted as a NOAEL/LOAEL interface, in that thyroid volumes increased slightly but significantly during the fifth week of daily administration of perchlorate at 12 mg/kg/day. Neither decreased T3/T4 nor increased TSH levels were observed in individuals who experienced these slight increases in thyroid volume. Hench, 12 mg/kg/day is a NOAEL for the thyroid-pituitary axis as normally defined, but a slight LOAEL for thyroid volume. For the reasons stated above, this endpoint would appear superior for RfD determination than the formerly utilized LOAEL of 1.4 mg/kg/day from the study of Stanbury and Wyngaarden (1952).

Given the sufficiency of the dosimetry database and knowledge of toxic mechanisms in both experimental and epidemiologic studies, it is recommended that a ten-fold safety factor is applied for extrapolating from subchronic human data to protection from chronic exposures, without need for an additional safety factor for database insufficiencies.

Male vs. Female Data

In the Burgi et al (1974) study of the effect of perchlorate on iodine secretion in five healthy volunteers, two were male and three were female.

No differences were noted between male and female volunteers. In the Brabant (1992, 1994, 1995) studies, all subjects were healthy males. In terms of perchlorate toxicities during therapy for hyperthyroidism, there have been no recorded differences between male and female patients.

It has been reported that hyperthyroidism (goiter) in humans is three times more prevalent in females than in males (Wartofsky, 1994). If this predilection for the disease were indicative of a greater sensitivity among human females to goitrogenic substances, including perchlorate, then this enhanced sensitivity would have to be taken into account while adjusting available NOAEL and LOAEL data for a safe human reference dose.

However, it appears that the various hyperthyroidisms, *viz.*, Graves' disease, which appear exacerbated in females are the result of sensitized female autoimmunity to TSH and other receptor proteins (Wartofsky, 1994). Females in general are many times more susceptible to all autoimmune diseases such as systemic lupus erythematosus and myasthenia gravis due to their more finely tuned T-lymphocyte system which must prevent potential fetal rejection during pregnancy (Hahn, 1994).

It is recommended that no extra safety factor is required for ensuring that females may be more sensitive than males to perchlorate toxicity and that the ten-fold safety factor for potentially sensitive populations takes any possible variation by sexes into account.

Other Potential Sensitive Populations and Target Organs

The population of hyperthyroid patients may be more sensitive to the actions of perchlorate than normal healthy individuals. However, rather than being adversely affected by perchlorate, it is beneficial to those afflicted by hyperthyroidism. No other more sensitive subpopulations, including fetuses, have been shown to exist. Although perchlorate passes the placenta and fetal thyroids enlarge upon daily exposure to perchlorate, this response is to levels comparable to those required for adult thyroid enlargement.

Clearly, perchlorate targets the thyroid gland by virtue of its competitive ability to inhibit iodine transport. All known toxicities of perchlorate to other target organs such as the exocrine and hematopoietic systems are probably mediated by thyrotoxicity and subsequent thyroxin depletion. In some described hypersensitivities in Graves' disease patients, these may include the effects of thyroxin depletion upon the immune system, especially inhibition of suppressor T-cells. The only non-thyroid-mediated toxicities of perchlorate require very high doses, in excess of

TABLE 8

Comparison of Human and Animal NOAEL and LOAEL Values for Perchlorate Toxicity to the Thyroid Gland

Reference	NOAEL (mg/kg/d)	LOAEL (mg/kg/d)
Studies in Normal Human Volunteers		
Brabant et al (1992)	12	
Brabant et al (1994)		12
Burgi et al (1974)		9.7
Shigan (1963)		2.9
Studies in Hyperthyroid Patients		
Stanbury & Wyngaarden (1952)	0.14	1.4*
13 Subchronic Studies		6.0
Studies in Experimental Animals		
Männistö et al (1979)	7.6	15.3
Shigan (1963)	0.25	2.0
Pflugfelder (1959)		20**

*An acute study with endpoints measured within hours.

**This was the lowest dose tested for thyrotoxicity in chickens.

It should be noted that all subchronic NOAEL and LOAEL values summarized above in normal humans, hyperthyroid patients and test animals are within an order of magnitude (2-20 mg/kg/day), except for the 0.25 mg/kg/day NOAEL of Shigan (1963) and the 0.14 mg/kg/day NOAEL of Stanbury and Wyngaarden (1952). However, the Shigan (1963) doses studied dropped from 2.0 to 0.25 mg/kg/day, with no doses in between. Obviously, higher NOAELs may have been observed in this study if intermediate doses between 2.0 and 0.25 mg/kg/day had been evaluated. The utility of the Stanbury and Wyngaarden study is addressed in the following section.

SECTION A5

DISCUSSION OF SAFETY FACTORS

In the subsections below, the relevance of the above-summarized data to establishing safety factors for the conversion of NOAEL and LOAEL data to an oral human reference dose for perchlorate is discussed.

Most Data Are From Subchronic Rather Than Chronic Studies

Although chronic data are available for Graves' disease patients treated with perchlorate, the more recent physiologic studies in human volunteers have all been terminated after a relatively brief exposure period (1-6 weeks). Brabant et al (1994, 1995) noted that it took longer than four weeks of exposure for his healthy volunteers to develop slightly increased thyroid volume, even though other cardinal signs of hyperthyroidism (T3/T4 diminution, TSH increase) had not occurred. Hence, it would appear that the NOAEL of 12 mg/kg/day perchlorate noted in the Brabant (1992) study is more precisely a NOAEL/LOAEL and similar to the LOAEL observed by Stanbury and Wyngaarden (1952) of 1.4 mg/kg/day, although the latter was derived during an acute study in one hyperthyroid patient.

The lowest LOAEL of 6 mg/kg/day identified from a review of subchronic studies comes from the brief review of Morgans and Trotter (1960) in which 6/180 hyperthyroid patients developed skin rashes, sore throats and gastrointestinal irritation after a few weeks treatment with perchlorate. These epithelial reactions in only 3 percent of treated patients may reflect immunoglobulin-mediated hypersensitivity to perchlorate in this small minority. The dose range of these patients was actually 400-1000 mg/day and body weights of the six patients who developed symptoms is not given. If body weights of the afflicted individuals were 50 kg rather than the 70 kg assumed for calculation of the 6 mg/kg/day LOAEL and if they had been exposed to 1000 mg/day rather than the 400 mg/day assumed, then this LOAEL might be more in the range of other values seen (≥10 mg/kg/day). Unfortunately, the raw data required for these calculations are not available.

Many of the other observed toxic effects of perchlorate seen after chronic administration of ≥10 mg/kg/day, such as agranulocytosis and aplastic anemia, are mediated by impairment of bone marrow which in turn is

grams/day, and are not relevant for determination of an oral reference dose.

Although the data suggest that some sensitive subpopulations (i.e., hyperthyroid patients) may benefit from administration of perchlorate, it is recommended that a ten-fold safety factor be applied in the development of an RfD to ensure protection over the range of human sensitivity.

DISCUSSION REGARDING THE ROLE OF THE THYROID GLAND IN MEDIATING PERCHLORATE INDUCED APLASTIC ANEMIA

Graves' disease is a common type of autoimmune hyperthyroidism often accompanied by bulging eyes and skin manifestations. It most commonly strikes women in their 30s or 40s. Since all known effects of Graves' disease are mediated by excess thyroxin production, perchlorate and other hypothyroid inducing agents have been utilized in the treatment of Graves' disease patients.

In the 1960s, several patients receiving ≥10 mg/kg/day perchlorate for Graves' disease therapy developed fatal aplastic anemias (Hobson, 1961; Johnson and Moore, 1961; Fawcett and Clarke, 1961; Krevans et al, 1962; Gjemdal, 1963; Barzilai and Sheinfeld, 1966). Given that thyroid carcinogenesis has not been seen in patients, aplastic anemia is obviously the most serious of perchlorate-induced human toxicities documented in the medical literature.

As outlined below, it appears that perchlorate induces aplastic anemia by the same mechanisms of its known ability to inhibit thyroxine formation by the thyroid gland and the known requirement of thyroxin for normal hematopoiesis.

Hematopoiesis and the Endocrinology of Aplastic Anemia

Hematopoiesis is a complicated orchestration of bone marrow to form all those varied cells which are found in blood, including erythrocytes (red blood cells), leukocytes (white blood cells) and platelets. Many hormonal reactions are important in controlling hematopoiesis, including endocrines, such as T3 (triiodothyronine) and T4 (thyroxin), which are produced outside the bone marrow, and cytokines, such as the interleukins, which are produced within bone marrow. Aplastic anemia is a potentially fatal condition in which bone marrow becomes deprived of almost every cell precursor for cells normally found in blood. The patient not only becomes anemic due to the lack of erythrocytes, but also immunosuppressed (due to the lack of lymphocytes) and at risk for internal bleeding (due to the lack of platelets).

According to Kelly Davis, M.D., Department of Endocrinology, University of Pennsylvania School of Medicine, who is a specialist in thyroid function, the thyroid gland does participate actively in the regulation of bone marrow development through elicitation of both T3 and T4, especially the former (Davis, 1996). According to Dr. Davis, adequate levels of triiodothyronine are essential for proper bone marrow development. Further evidence gained from leukemia cells supports this contention (see following section).

Thyroxin-Receptors in Bone Marrow Stem-Cells

Two genes on human chromosomes 17 and 3 (TR-*alpha* and TR-*beta*, respectively) control the formation of thyroid receptors (TR) on all thyroxin-sensitive cells. These TR proteins each have T3/T4-binding and DNA-binding domains, the latter termed TRE for "thyroid response element."

Target genes for several functions are not activated until their respective TRE sequences are activated by thyroxin binding, including:

- Thyroid stimulating hormone (TSH);
- Prolactin (required for lactation);
- Growth hormone (required for bone growth); and
- Hematopoietic genes.

When bound by T3 or T4, the TR proteins migrate from outside the cell membrane to the nucleus and initiate gene action by binding to the appropriate TRE sequences. The highest concentrations of TR receptors are found in the pituitary, brain, liver, heart, kidney and bone marrow.

Much of the specific information about TR proteins in bone marrow cells and responsive hematopoietic genes has been gained from a study of leukemia cells. The gene which synthesizes TR proteins on the cell surface of bone marrow cells has been recognized as *c-erbA*, whose product binds T3 and T4 with high affinity (Sap et al, 1986; Weinberger et al, 1986). The *c-erbA* TR binds T3 ten times more assiduously than T4. The *c-erbA* TR functions much as a steroid receptor in its mechanism of hormonal binding, cellular incorporation and nuclear control (Eisenman,1989).

Conclusions Regarding Perchlorate-Induced Aplastic Anemia

The strongest triiodothyronine (T3) and thyroxin (T4) receptors in the human body are found in bone marrow stem cells. If the thyroid gland is impaired in its ability to produce adequate levels of T3 and T4, these bone marrow stem cells stop differentiating and virtually all bone marrow lineages cease. Hence, the most likely mechanism for aplastic anemia induction by perchlorate is equivalent to that for thyroid carcinogenesis (i.e., a mechanism which requires chronic administration of high doses for prolonged periods) and is not of use in establishing safe levels from environmental exposure.

SECTION A7

OVERALL CONCLUSIONS REGARDING PERCHLORATE TOXICITY

As evidenced in several previous reviews (ECAO, 1992; ICF, 1993; PSG, 1994) and herein, the database for perchlorate toxicity is replete with NOAEL and LOAEL values from normal human volunteers, hyperthyroid patients and experimental animals. The best dose-response data available from five sources of information are remarkably consistent:

- Normal human volunteers do not demonstrate any thyrotoxicity to 12 mg/kg/day perchlorate for four weeks of administration daily and only after the fifth week show a slight increase in thyroid volume (Brabant et al, 1992, 1994, 1995).

- Burgi et al (1974) determined that 9.7 mg/kg/day was a LOAEL in healthy humans for purging radioiodine from the thyroid.

- The lowest LOAEL and highest NOAEL in a hyperthyroid patient treated with perchlorate were 1.4 and 0.14 mg/kg/day, respectively (Stanbury and Wyngaarden, 1952).

- Rats treated subchronically with a range of perchlorate doses show a NOAEL at 7.6 mg/kg/day and a LOAEL at 15.3 mg/kg/day (Männistö et al, 1979).

- The LOAEL for blocking radioiodine uptake in rat and rabbit thyroids is 2.0 mg/kg/day (Shigan, 1963).

That rodent and human data are consistent should not be surprising in that the pituitary-thyroid axis is similar among all mammals. Furthermore, in that perchlorate acts directly to block iodine uptake without any requirement for metabolism, there is no need to adjust rodent doses to human doses through a scaling factor for surface area/body weight ratios.

Males and females appear to have equal sensitivities to perchlorate and no other sensitive populations, except hyperthyroid cases who would be benefited by perchlorate exposure, have been identified. All additional organ site sensitivities may be attributed to indirect effects of perchlorate on inhibition of thyroxin synthesis by the affected thyroid gland target organ site. Perchlorate is neither a reproductive toxin nor is it genotoxic.

It is recommended that, given the amount of concordant data available, the Brabant et al NOAEL/LOAEL value of 12 mg/kg/day determined in normal humans be adopted as the basis for a reference dose for application to human risk assessment. Given the plethora of human data available and its consistency with data derived from test animals along with the well understood mechanism of perchlorate toxicity, it is recommended that the following two safety factors be applied:

- Ten-fold for the potential range of human sensitivity; and
- Ten-fold for extending subchronic human data to protection from chronic exposure.

Application of these two 10-fold safety factors to the human NOAEL/LOAEL value of 12 mg/kg/day yields a recommended human oral reference dose of 0.12 mg/kg/day. This RfD of 0.12 mg/kg/day has been utilized previously in this document to characterize potential risks to human receptors on the Aerojet site from exposure to perchlorate in groundwater at 4 mg/L, showing that this level is without adverse health impact.

GLOSSARY OF TERMS

GLOSSARY OF TERMS

Some of the terms used in this report are technical and may be unfamiliar to the informed layperson. The following glossary of terms may be helpful.

Amylolytic activity of the parotid - Parotid glands, among other functions, excrete enzymes which break down amyloid, the substance which accumulates in neural tissue (in excess in Alzheimer's patients).

Aplastic anemia - A potentially fatal condition in which bone marrow becomes deprived of almost every cell precursor for cells normally found in blood. The patient not only becomes anemic due to the lack of red blood cells, but also immunosuppressed (due to the lack of white blood cells) and at risk for internal bleeding (due to the lack of platelets).

Agranulocytosis anemia - A type of anemia characterized by a lack of granulocytes, a type of white blood cell containing many granules.

Autoimmune hyperthyroidism - Enlargement of the thyroid gland due to antibodies to TSH-receptors which act as thyroid stimulating hormone (TSH).

Blastocyst - That stage of the vertebrate embryo in which an inner cavity (endoderm) can first be distinguished.

Carbimazole - A drug which acts to inhibit growth of the thyroid gland and, hence, used in the treatment of hyperthyroidism.

Cytokines, such as the interleukins - Produced within bone marrow and are necessary chemical signals for normal white blood cell development.

Endocrinologist - A medical specialist who studies endocrine glands, such as the thyroid, pancreas or pituitary glands.

Epidemiologic - That type of study which determines the association between exposure and diseases in human populations.

Fetotoxic dose - The dose which is toxic (lethal) to the developing fetus.

Follicular adenomas - Benign tumors of the thyroid gland which develop in the follicles (i.e., those nested regions which synthesize thyroid hormones).

Follicular cell carcinogenesis - Development of cancer within the follicles of the thyroid gland (see above).

Gastrointestinal - Pertaining to the central alimentary canal of the body, also known as the G.I. tract.

Goitrogenesis - Goiter (enlarged thyroid gland) formation.

Goitrogens - Those substances which induce goiters by blocking iodine uptake.

Genotoxicity - Toxic to genes, either mutagenic (change DNA) or clastogenic (change chromosomes).

Gravid test animals - Mice or rats which are pregnant.

Hematopoiesis - The synthesis in bone marrow of all cells eventually found in blood, including erythrocytes (red blood cells), leukocytes (white blood cells) and platelets.

Hematopoietic genes - Those genes which control the formation of blood cells in bone marrow.

Hyperthyroidism - Enlarged thyroid gland, synonymous with goiter.

Immunoglobulin-mediated hypersensitivity - Being allergic to one's own tissue(s) because of the presence of an antibody which reacts with self.

Methemoglobulinemia - The presence of reduced iron in the hemoglobin molecule of red blood cells is incapable of carrying sufficient oxygen.

Myasthenia gravis - An autoimmune disease in which antibodies react against acetyl choline receptors of nerve cells, disrupting nerve function.

Nephrotic syndrome - A disease of the kidneys which allows too much protein to accumulate in urine.

Nongenotoxic - Not toxic to genes, unreactive to either DNA or chromosomes.

Percutaneous - Into the skin.

Perturbation - Disturbance.

Pituitary-thyroid axis - The communication links and delicate balance between pituitary and thyroid gland in controlling the function of the latter. If insufficient thyroid hormones are produced by the thyroid, the

pituitary senses this deficit and signals the thyroid through thyroid stimulating hormone to manufacture more thyroxin.

Plethora - Multitude, sometimes excessive.

Prolactin - A pituitary hormone that is required for lactation (milk production) by mammals.

Propylthiouracil - A substance which, like perchlorate, blocks iodine uptake and, hence, in sufficient concentrations, induces hyperthyroidism.

Prototypic - Of primary example.

Radioiodines - Iodines which have unstable nuclei and, hence, give off radiation (normally in the form of beta-particles).

Systemic lupus erythematosus - An autoimmune disease which is characterized by antibodies to one's own chromosomes.

Thyroid hyperplasia - Excessive growth of the thyroid gland which, if unabated, results in a goiter.

Thyroid-pituitary axis - See Pituitary-thyroid axis.

Thyrocyte - That epithelial cell of the thyroid which is sensitive to thyroid stimulating hormone.

Thyroglobulin - Equivalent to thyroid stimulating hormone (TSH), made by the pituitary gland.

Triiodothyronine (T3) - A thyroid hormone containing three atoms of iodine.

Thyroxin (T4) - A thyroid hormone containing four atoms of iodine.